FACTORS AFFECTING THE UTILISATION OF SILAGE NITROGEN BY RUMINANTS

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ERRATUM Throughout, for 'Macrae', read 'MacRae'.

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DECLARATION

With due regard to the assistance which has been acknowledged on page (i), this thesis was written by, and describes work conducted by, Michael James Proven.

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ABSTRACT

Eight ryegrass silages, which differed in their contents of dry matter, nitrogen and in the pre-ensiling treatments which were used, were fed to sheep cannulated at the rumen and abomasum. Pelleted, dried grass was fed throughout to confirm that inter-experiment comparisons could be safely made. Dietary digestibility was measured by total faecal collection and diurnal variation in ruminal metabolism was monitored. Passage of the components of digesta at the abomasum was determined by reference to the dilution of dual phase markers (tris (1, 10 phenanthroline) ruthenium (II) chloride and the chromium complex of ethylenediamine tetraacetic acid) which were continuously infused into the rumen. The contribution of microbial nitrogen to the total flow of nitrogen at the abomasum was assessed by using nucleic acid as an endogenous microbial marker.

All silages were well preserved. Fermentation was restricted by pre-treatment with formic acid (3.0 and 3.4 l t^{-1}), a mixture of formic acid and formalin (4:1, 4.5 l t^{-1}) or by wilting. Proteolysis was also reduced by these treatments, with wilting to high levels of dry matter (416 and 438 g kg^{-1}) having the greatest effect.

There was a tendency for higher concentration of nitrogen in silage to increase the apparent digestibility of the component. Silages with high concentrations of

nitrogen therefore had disproportionately high contents of digestible crude protein.

In absolute terms, the peak ruminal ammonia concentration was higher when dried grass, rather than silage, was fed (mean = 349 and 239 mg ammonia N l^{-1} respectively. However the ruminal ammonia concentration before feeding and the content of nitrogen in the diet were both lower, and the ration was eaten more slowly, when silage was fed. Thus the consumption of silage induced bigger changes in ruminal ammonia levels per unit intake of nitrogen, probably reflecting the soluble nature of a large proportion of the nitrogenous components of the silages.

The mean values for the proportion of digestible organic matter apparently digested in the rumen (0.614) and the efficiency of synthesis of microbial nitrogen (26.2 g N kg^{-1} DOMR, 0.91 g N MJ^{-1} ME intake) were similar to those which had been reported previously for silages. However, the mean degradability of silage nitrogen between the mouth and the abomasum (0.47) was lower than many previous estimates.

When silage was fed, the flow of non-ammonia nitrogen at the abomasum was negatively related to the concentration of nitrogen in the dietary digestible organic matter. This reduced efficiency of transfer of nitrogen from the diet to the small intestine at high dietary concentrations has been established before for dried diets, but not for silages.

1. INTRODUCTION

Grass is capable of producing greater quantities of nutrients for ruminant livestock than are most other crops grown in the United Kingdom (Gordon, 1980). The relative costs of cereal-based concentrate rations and fresh grass of equivalent nutritive value suggest that the maximum possible use should be made of the latter (Harvey, 1982). The seasonality of grass growth in the west maritime climate of the British Isles requires that excess spring and summer production be conserved for feeding in the winter (Sullivan, 1973), and the use of the ensilage process to achieve this has increased in recent years (Wilkins, 1975). Despite the costs involved in the silage-making process, forage conserved in this manner is still a less expensive provider of metabolisable nutrients than are concentrates (Harvey, 1982).

Recent attempts to improve the predictive accuracy of conventional systems for evaluating the protein requirements of ruminant animals and the ability of different foods to meet these requirements, have led to the development of systems with a more sound physiological background (ARC, 1980; Tamminga, 1982; ARC, 1984). Because of their greater dependence on detailed study of the metabolism of dietary nitrogen, these newer systems have been accompanied by developments in techniques for assessing the passage of nutrients at various points in the gastrointestinal tract and the degradation of dietary nitrogen in the reticulo-rumen (Lazenby, 1978).

Despite its undoubted importance to livestock farming in the United Kingdom, and probably because of the heterogenous nature of the material, silage has been the subject of a disproportionately

low number of experiments investigating the parameters required by the new evaluation systems (ARC, 1980, 1984).

In the present series of experiments the digestion and metabolism, by sheep, of a diverse group of eight grass silages was studied. The information provided by these studies could be expected to contribute to and enlarge the database of values needed to refine the newer systems for evaluating dietary protein (ARC, 1980, 1984).

2. REVIEW OF LITERATURE

2.1 THE ENSILAGE PROCESS

2.1.1 Introduction

In the late nineteenth century, silage was described as "green herbage which is placed in a silo in such a way as to get rid of and afterwards permanently exclude, the destroying oxygen of the atmosphere" (Ministry of Agriculture, Fisheries and Food, 1977). Now with the benefit of modern understanding of the biochemical and microbial process involved, a more satisfactory definition of silage is "the material produced by the controlled fermentation of a crop of high moisture content" (McDonald, Edwards and Greenhalgh, 1973; McDonald and Whittenbury, 1973). Others (Crawshaw, 1977; Thomas, Kelly and Chamberlain, 1980) have used less broad definitions and consider silage to be fresh forage, preserved from further degradation by the action of the acid products of natural microbial fermentation of the plant's carbohydrates. Such a definition would, strictly speaking, exclude forages stored at low dry matter, where fermentation has been modified or restricted (even to the extent of being virtually abolished) by the application of an exogenous additive (see Section 2.1.3.4 below). Such materials are universally referred to as silages, and the definition of McDonald et al (1973) remains the more general and satisfactory. Some workers have felt that the compositional differences between a silage treated with, for example, high levels of formaldehyde and an untreated silage are of such significance that the term "non-fermented silage", defined as stored forage with total fermentation acids present at <50 g kg^{-1} dry matter (DM), should be employed (Wilson and Wilkins, 1974).

The conservation of forage by the process of ensilage was known to the ancient Egyptians some 3000 years ago, but the technique did not gain widespread acceptance in Britain until after the Second World War (Thomas et al, 1980). Green crop preservation and storage is an important activity in modern grassland agriculture, stimulated primarily by the seasonality of grass produced in temperate countries which requires that surplus production in the summer be conserved for feeding in the following winter (Sullivan, 1973). In most farming systems, the choice of conservation system is between silage and field dried hay, the capital and energy costs of alternative drying systems being prohibitive (MAFF, 1977). In temperate northern and western Europe, hay-making is at a disadvantage because of its reliance on warm, dry weather (Sullivan, 1973). Thus, despite the technique's higher demands on 'support' energy (Thomas et al, 1980) the quantitative importance of silage has increased, at the expense of hay-making, in recent years (Wilkins, 1975).

2.1.2 Natural silage fermentation

The biochemical changes occurring during ensilage result from the activities of plant enzymes, lactic acid bacteria, clostridia, enterobacteriaceae and yeasts (McDonald et al, 1973; McDonald and Whittenbury, 1973; Ohshima and McDonald, 1978; McDonald, 1980).

The actions of plant enzymes have been elucidated in experiments in which sterile grass has been cultured and ensiled (Kemble, 1956), or where the microbial population has been killed by gamma-irradiation before ensiling (Wilkins, 1975; Ohshima and McDonald, 1978). Under aerobic conditions, either in the field or before

all the oxygen in the consolidated ensiled mass has been consumed, plant respiratory enzymes oxidise sugars producing heat, carbon dioxide and water (McDonald, 1980). The soluble carbohydrate available for subsequent fermentation is thus reduced. Hydrolysis of heteropolysaccharides may also occur, having the opposite effect to the oxidation described above and increasing the availability of soluble carbohydrates (Carpintero, Holding and McDonald, 1969; McDonald, 1980).

Rapid and extensive breakdown of plant protein also occurs in the first few days of ensiling and within 24 h the non-protein N content of herbage can increase from less than 200 to more than 400 g kg^{-1} total N (McDonald, 1980). Kemble (1956) demonstrated that the non-protein N content of ensiled sterile grass had risen to 600 g kg^{-1} total N after 16 d. The main end products of these proteolytic reactions are peptides and free amino acids (McDonald, 1980), but decarboxylation of some amino acids can occur (Ohshima and McDonald, 1978). The extent of deamination of amino acids is difficult to assess since although the concentration of ammonia does not rise, the facility for evolved ammonia to be recombined with alpha-ketoglutarate to form glutaric acid exists (Ohshima and McDonald, 1978).

2.1.2.1 Lactic acid bacteria

The production, under anaerobic conditions, of organic acids by naturally occurring bacteria, is the principal means by which silage preservation is effected (Thomas et al, 1980). Normally, the most important acid is lactate and the microorganisms responsible for its production are collectively known as lactic acid bacteria (McDonald et al, 1973; McDonald, 1980). Bacteria whose sole

metabolic product, following sugar fermentation, is lactate are termed homofermentative species. Pediococcus acidilactici, P. cerevisiae, P.pentosaceus and Lactobacillus plantarum are usually the dominant organisms of this type (McDonald, 1980). Heterofermentative lactic acid bacteria, producing lactate and acetate or alcohols, are also found and include Leuconostoc spp. and Lactobacillus brevis (McDonald, 1980). Homo- and hetero-fermentative lactic acid bacteria are facultative, not obligate, anaerobes and in the early, aerobic, stages of ensilage all species produce a mixture of acetate, acetoin, formate, lactate, pyruvate, carbon dioxide and water. There is evidence that, initially, homofermentative species are dominant but that as fermentation proceeds, heterofermentative organisms gradually become numerically more important, and that this may be, in turn, connected with the latter group's greater tolerance of acetate (McDonald and Whittenbury, 1973; McDonald, 1980). The production of normally fermented silages, in which acetate, rather than lactate, is the dominant fermentation acid, has been reported (Barry, Cook and Wilkins, 1978) and such silages have been recognised in classification systems (McDonald and Edwards, 1976). Acetate may also be produced early in the ensilage process by the action of Enterobacteriaceae, facultative anaerobes which compete with lactic acid bacteria for fermentable sugars.

The rapidity with which the pH of the ensiled material falls depends not only on the production of acids by bacteria, but also on the buffering capacity of the crop (McDonald and Whittenbury, 1973). Initially, this resistance to pH change

is attributable to endogenous plant organic acids (McDonald, 1980; Thomas et al, 1980). It differs between species (Carpintero et al, 1969) and, within plant species, between different stages of maturity (Henderson and McDonald, 1976). The latter workers demonstrated that buffering capacity decreased with increasing maturity in three cultivars of ryegrass examined at the beginning of ear emergence, at 50% ear emergence and at 100% ear emergence. During ensilage, the levels of plant organic acids fall, but the formation of lactate, acetate and other fermentation acids means that buffering capacity may increase by three- or four-fold (McDonald and Whittenbury, 1973).

The characteristic light brown/green colour of silage is caused by the magnesium-free pigment phaeophytin, produced by the action of organic acids on chlorophyll (McDonald and Whittenbury, 1973).

Lactic acid bacteria are considered to be non-proteolytic, but different groups of these organisms have varied abilities to deaminate free amino acids (Ohshima and McDonald, 1978; McDonald, 1980). The homofermentative L. plantarum and Pediococcus spp.can deaminate serine and arginine to pyruvate and ornithine respectively whereas L. brevis deaminates L-arginine only (Ohshima and McDonald, 1978). The former group are active only in the first few days following ensiling but the activity of L.brevis persists for at least 35 d (Ohshima and McDonald, 1978).

Despite the fact that lactic acid bacteria have no proteolytic activity, researchers have generally observed that the soluble, non-protein, fraction of the total N of ensiled material increases with increasing ensilage period. Thus Kemble (1956), when ensiling

young perennial ryegrass (Lolium perenne, cv. S24) with an inoculum of Lactobacilli and glucose solution, found that the attainment of pH 3.9 within 3 d did not prevent the water-soluble N fraction rising to a level of 600 g kg^{-1} total N. The level of ammonia N was low (30 g kg^{-1} total N), but the total water-soluble N proportion continued to increase slowly with increased length of the ensilage period. Kemble also demonstrated that the levels of all amino acids except alanine were less than would have been expected if proteolysis had been unaccompanied by subsequent metabolism of the liberated acids. Bergen, Cash and Henderson (1974) observed proteolysis continuing several weeks after ensiling chopped whole maize (Zea mais). Water-soluble N increased from the level at harvest of 132 g kg^{-1} total N to 365 g kg^{-1} total N within 12 h. Over the following 20 d, the level rose slowly to 416 g kg^{-1} total N. These results showed, however, the overwhelming importance of the initial, plant enzyme-catalysed, proteolysis. Assessments of the proteolytic activity of silage samples, made by measuring the amount of non-protein N produced when a sample of silage was incubated with casein, indicated that this had decreased to less than 25% of its original value five days after ensiling.

2.1.2.2. Clostridia

Members of the Clostridium genus of microorganisms are obligate anaerobes (McDonald, 1980) which are actively saccharolytic, fermenting sugars and lactate to butyrate and small quantities of ethanol, butanol and formate (McDonald et al, 1973; McDonald, 1980; Thomas et al, 1980). They also exhibit proteolytic activity, and degrade the resulting amino acids by a variety of deamination, decarboxylation and coupled oxidation-reduction (Stickland) reactions (McDonald et al, 1973; Ohshima and McDonald, 1978; McDonald, 1980).

Clostridia are conventionally considered to be important in silage fermentation only when the pH resulting from the fermentation of water-soluble carbohydates by lactic acid bacteria has been insufficiently low to restrict their growth (MAFF, 1977). The required pH varies with the dry matter concentration in the ensiled crop since the higher osmotic pressure of dry material also inhibits the growth of these microorganisms (Marsh, 1979). Inhibition of clostridia may occur at a pH as high as 5.0 when the dry matter content of the crop is $\geqslant$300 g kg^{-1}, but clostridial activity may not even be suppressed at the accepted 'safe' limit of pH 4.0 if crop dry matter is <150 g kg^{-1} (McDonald and Whittenbury, 1973; Crawshaw, 1977). The 'secondary fermentation' which results produces elevated levels of butyrate which, being a less strong acid than lactate or acetate, causes a rise in the pH of the ensiled mass to 5.0 - 6.0 (McDonald and Edwards, 1976). Decarboxylation reactions result in the formation of amines such as tyramine, tryptamine, histamine and putrescine as well as gamma-amino butyrate (McDonald, 1980; Thomas et al, 1980). Silages in which significant clostridial activity has occurred are further characterised by high levels of ammonia (McDonald and Edwards, 1976). Kemble (1956) attempted, on a laboratory scale, to produce silage from L. perenne with the characteristic attributes of clostridial fermentation. He therefore inoculated the grass with clostridia before ensiling. The sugars in the grass were depleted before a marked pH drop was obtained, proteolytic and deaminatory activity continued and, at the end of the 147 d ensilage period, the ammonia N level was 600 g kg^{-1} total N. In a similar silage where inoculation

with Lactobacilli spp. had rapidly reduced the pH to 3.9, the ammonia N level was only 30 g kg^{-1} total N.

McDonald (1980) has pointed out that the term 'secondary' may be misleading when referring to the activities of clostridia in silage. Although they are obligate anaerobes, the attainment of anaerobic conditions in a consolidate crop of low dry matter is rapid, and certain strains (eg Clostridium sporogens and C. bifermentans) multiply with other bacteria and contribute to the early exponential growth (Ohshima and McDonald, 1978; McDonald, 1980). Thus even silages whose fermentation quality is considered to be 'good' (Ekern, Saue and Vik-Mo, 1975) or typically 'lactate' (McDonald and Edwards, 1976) have low, but appreciable, levels of butyrate (<1.0 g kg^{-1} DM) and ammonia (<80 g kg^{-1} total N).

2.1.2.3 Enterobacteriaceae and yeasts

Enterobacteriaceae occurring on grass and in silage are Gram negative facultative anaerobes (McDonald, 1980). They are thought to be active only in the early stages of the ensilage process, since their intolerance of low pH means that their activity is greatly reduced once lactate levels rise (McDonald, 1980). Their principal fermentation product is acetate, although carbon dioxide, hydrogen, lactate and alcohols are also produced (McDonald, 1980). Enterobacteriaceae exhibit, at most, only weak proteolytic activity although they can decarboxylate and deaminate free amino acids (McDonald, 1980).

Yeasts with both fermentation and respiration capabilities have been found to occur in silages (McDonald, 1980). They are generally considered to be an undesirable minority group

in the silage micro-flora, being associated with silages where secondary aerobic deterioration occurs, and whose main fermentation product, ethanol, contributes little to the preservation process (McDonald, 1980). Yeasts are not inhibited by lactic acid (McDonald, 1980) and may be differentially encouraged when an acid additive with anti-bacterial action is applied (Henderson and McDonald, 1971).

2.1.2.4 Classification of silage quality

The classification of the fermentation quality of silages is an area where few definable standards exist. McDonald and Edwards (1976) recognised five broad categories of silage. Lactate and acetate silages exhibited low pH (c. 3.7 - 4.2), ammonia N (c. 80 g kg^{-1} total N), protein N (c. 230 g kg^{-1} total N) and water-soluble carbohydrate (c. 10 g kg^{-1} DM) levels, and differed only with respect to the relative concentrations of the fermentation acids lactate and acetate. Butyrate silage had a higher pH (c. 5 - 6), butyrate (c. 35 g kg^{-1} DM) and ammonia N (250 g kg^{-1} total N) levels. Wilted and chemically restricted silages, (see Section 2.1.3.6 and 2.1.3.4 below) in which the formation of fermentation acids was restricted and signficant amounts of plant proteins and water-soluble carbohydrates were preserved, were also recognised. Most such systems rely on the measurement of deleterious butyrate and ammonia N levels to categorise silages (Ekern et al, 1975). McDonald and Whittenbury (1973) presented a table summarising the categorisation systems used by a number of authors (Table 2.1). This set of data clearly illustrates the variation in such systems and emphasises the heterogeneity of ensiled material.

Table 2.1 Observed variations in silage categorisation systems (after McDonald and Whittenbury, 1973)

Quality Classification	pH	Butyric acid concentration (g kg^{-1} DM)	Volatile N concentration (g kg^{-1} total N)
Very good	4.2	<1.0	50 - 80
Very good	-	<1.0	<125
Good	<4.2	<1.0	<80
Good	<4.2	<2.0	<80
Good	-	1.1 - 2.0	125 - 150
Satisfactory	<4.2	<1.0	<110
Medium	4.3 - 4.5	3.0 - 5.0	90 - 115
Medium	-	2.1 - 3.0	151 - 175
Poor	>4.5	>5.0	50 - 80
Bad	-	3.1 - 4.0	176 - 200
Very bad	-	>4.0	>201

2.1.3 Manipulation of the natural ensilage process

2.1.3.1. Introduction

The attributes of a 'well fermented' silage, in which preservation has been effected by natural fermentation of the crop's water-soluble carbohydrates to lactate, have been identified and categorised (Mcdonald and Whittenbury, 1973; Ekern et al, 1975; McDonald and Edwards, 1976). It has also been recognised that the combination of low dry matter, low water-soluble carbohydrate and high buffering capacity found in certain forages, for example lucerne (Medicago sativa) (Carpintero et al, 1969)

and Cocksfoot (Dactylis glomerata) (McDonald, Stirling, Henderson and Whittenbury, 1964), or brought about by heavy applications of nitrogenous fertiliser (Ekern et al, 1975), may lead to a natural fermentation where saccharolytic, proteolytic Clostridium spp. become the dominant organisms. Attempts have therefore been made to manipulate the fermentation process (Virtanen, 1933; McDonald and Whittenbury, 1973; Ekern et al, 1975; McDonald, 1980). The methods employed in these attempts may conveniently be divided into two broad groups. The first grouping embraces all techniques where substances are added to the crop prior to ensiling. This is a diverse group, which includes chemicals with more or less specific antimicrobial effects (Virtanen, 1933; Davis, Dulbecco, Eisen and Ginsberg, 1973; Wilkins, Wilson and Woolford, 1973; Henderson and McDonald, 1976; Crawshaw, 1977; Woolford, 1978), supplementary water-soluble carbohydrates for indigenous microbes (Carpintero et al, 1969; Ekern et al, 1975; Crawshaw, 1977) and inocula of additional hetero- or homo-fermentative lactic acid bacteria (McDonald et al, 1964; Carpintero et al, 1969; Carpintero, Henderson and McDonald, 1979), These pre-ensiling treatments are described in Section 2.1.3.2 below. The second group is simpler, embracing those techniques designed to increase the dry matter content of the crop between cutting and ensiling (Henderson and McDonald, 1976; Marsh, 1979) (see Section 2.1.3.6 below).

Initially, 'manipulation' of silage fermentation was designed to provide conditions to ensure that a 'natural', well fermented, silage was produced (Ekern et al, 1975). However as early as the 1930's in Europe, consideration was being given to the use

of additives which would restrict the activities of all indigenous microorganisms and produce a preserved material in which anaerobic fermentation had been restricted (Virtanen, 1933). More recently, attention has moved from the fermentation quality *per se* of silage, and has focussed instead on the nutritive value of the final, conserved, product (Ohshima and McDonald, 1978; McDonald, 1980). There have been numerous reports that the products of carbohydrate fermentation can reduce the voluntary dry matter intake of silage by ruminants. Work published at the beginning of the last decade (McLeod, Wilkins and Raymond, 1970) indicated that the acid content of well-fermented silage might suppress the voluntary intake of the material by ruminants. A negative correlation between the dry matter intake of silage and the concentration of individual fermentation acids, fermentation acids collectively and hydrogen ions has since been reported by several workers (Wilkins, Hutchinson, Wilson and Harris, 1971; Brown and Radcliffe, 1972; Wilkins *et al*, 1973; Wilkinson, Wilson and Barry, 1976; Crawshaw, 1977). The mechanisms of such intake regulation are, however, not fully understood and intra-ruminal infusions of lactate or acetate have not invariably reduced forage intake in ruminants (Thomas *et al*, 1980). There have also been suggestions that the soluble N products of the proteolytic activities of plant and microbial enzymes, and more particularly the products of decarboxylation and deamination by *Clostridium* spp. may play a role in the regulation of silage intake (Wilkins *et al*, 1973; Bergen *et al*, 1974). Again, however, little success has been achieved when attempting to reduce intake by intraruminal infusion of these compounds (Thomas *et al*, 1980).

Dissociation of the nutritive value of silage from its fermentation quality presents problems when attempting to assess the relative merits of forage conservation and feeding systems (Ekern et al, 1975). Observations of intake/fermentation correlations have led to increased interest in the use of pre-ensiling treatments designed to preserve harvested forage without, or with greatly reduced, formation of fermentation acids by bacteria. Whether such treatments produce 'controlled fermentation' or 'non-fermented' silages may depend on the application level and also on the classification system adopted (see Section 2.1.2.4 above).

2.1.3.2. Silage additives

Regional variations, not only in type of crop ensiled, but also in the employment of wilting and additive application techniques are seen throughout continental Europe and Britain (Ekern et al, 1975; Wilkins, 1975). The use of additives in northern European countries (Denmark, Sweden, Norway, Finland) is virtually universal (Ekern et al, 1975; Wilkins, 1975), such extensive use probably being stimulated by the vagaries of Scandinavian weather and by the particular grass species cultivated there (Ekern et al, 1975). Throughout the rest of Europe the use of chemicals as aids to the natural ensilage process is less widespread - in many areas this is because forage crops are wilted to relatively high dry matter contents (400 - 500 g kg^{-1} fresh weight) before ensiling - but in Britain and Ireland in particular the use of additives is increasing (Wilkins, 1975). Recent figures indicate that in Europe, out of a total production of 85 million tonnes of silage, 25 million tonnes (29%) were

ensiled after treatment with formic acid additives alone (Drysdale, 1979). A recent ADAS-sponsored survey (Minister, 1978) in which 1,091 silage samples from the 1976 crop in England and Wales were analysed found that 545 (50%) had received no additive treatment at all, whereas 219 (20%) had been treated with formic acid.

Although general consensus exists concerning the classification of silage additives, agreement on the specific categories, and to which category specific additives should be allocated, is not universal. McDonald and Whittenbury (1973) and Crawshaw (1977) recognised two categories: "stimulants" and "inhibitors". Owen (1971) identified two broad groupings: additives primarily designed to alter fermentation patterns and additives primarily designed as nutrient supplements. Since Owen stated that members of the second group could be added after fermentation they are largely outwith the scope of this review. Within his first group Owen discussed silage additives in three categories - sterilants, direct acidifiers and lactic acid stimulants. Wilkinson et al (1976) also recognised three additives classes: (1) those which promoted fermentation by provision of additional water-soluble carbohydrate, (2) those which prevented or severely restricted fermentation and (3) those which controlled fermentation by providing conditions which favoured Lactobacilli spp. and inhibited clostridial activity. The "Mcdonald/Crawshaw" system and that of Wilkinson and his co-authors are not irreconcilable since McDonald and Whittenbury (1973) included broad spectrum and selective sub-groupings in their "inhibitor" category, these two sub-groupings corresponding to the second and third

classes in the "Wilkinson" system.

While it must be recognised that all additive types are applied with control of fermentation, to a greater or lesser extent, in mind, some ordering would be beneficial. In the following sections the system advocated by Wilkinson et al (1976) will be used and when the categorisation of a particular additive depends on its application rate a relevant entry will be made in each appropriate additive class. This normally arises with certain materials which, although primarily intended to operate as class 3 additives, will function in a class 2 mode if present in high enough concentrations.

2.1.3.3 Class (1) additives -supplementary water-soluble carbohydrate

In one of his original papers advocating the use of mineral acid treatments for silage production (see below), Virtanen (1933) stated that successful results had been achieved by adding molasses to crops with which difficulties had been experienced during additive-free ensiling. The mode of action of a water-soluble carbohydrate additive is to supply additional energy substrate for endogenous lactic acid bacteria, resulting in rapid production of lactic acid, a concomitant rapid fall in pH and thus the attainment of an intra-silo environment unsuitable for saccharolytic and proteolytic clostridial activity. Proliferation of lactic acid bacteria in a few days has been observed within molasses treated silages, similar events not occurring until the second week after silo filling in corresponding control silages (Owen, 1971). This dependence on natural fermentation processes is perhaps the first disadvantage of molasses as an

additive (particularly when compared with class 2 and class 3 additives) and Virtanen (1933) cited the necessity for the provision of a watertight and airtight silo as being the major disadvantage of such assisted natural silage making. Now, the relatively large amounts of additive required to achieve a demonstrable effect are recognised as the biggest single disadvantage (Carpintero et al, 1969; Crawshaw, 1977).

In comparative, pilot-scale silo, experiments McDonald et al (1964) investigated interactions between molasses addition, crop type (Italian ryegrass (L. multiflorium) and Cocksfoot (D. glomerata), water-soluble carbohydrate contents 162 and 43 g kg^{-1} DM respectively) and level of inoculation with lactic acid bacteria. The molasses, which had a soluble sugar concentration of 456 g kg^{-1} DM was applied at a rate of approximately 18.5 kg t^{-1} ensiled as a 50% w/w solution. The workers concluded that at high water-soluble carbohydrate levels no beneficial response to molasses addition was evident since the control ryegrass silage had a low pH (in the range 3.9 - 4.1) with high lactate (100 g kg^{-1}DM) and low butyrate (2 g kg^{-1} DM) levels. With D.glomerata however, molasses alone and molasses with lactic acid bacteria inoculum improved the fermentation quality of the silage.

In later experiments at the same laboratory Carpintero et al(1969) demonstrated improved fermentation of lucerne ensiled in 1.2 kg capacity laboratory silos with molasses added at the rate of 40 kg t^{-1}. Such high water-soluble carbohydrate levels were deemed necessary so that high lactic acid concentrations could be achieved - a prerequisite for pH reduction in the lucerne

plant which has a high buffering capacity.

2.1.3.4 Class (2) additives - restriction of fermentation

2.1.3.4.1 Formaldehyde

The use of formaldehyde (usually applied as a 37 - 40% w/v aqueous solution, known as formalin) as a preservative of harvested forage crops has been investigated at least since the early part of the 20th century. Virtanen (1933) concluded from "earlier work" that formalin and other sterilants would see no practical application in silage making. With enhancement of fermentation quality firmly entrenched as the goal for all silage additives, antimicrobial agents were generally ignored but the preliminary work of McLeod et al (1970) and the recognition that the nutritive value of fermented forage was perhaps limiting animal performance (Hinks, Edwards and Henderson, 1976; Crawshaw, 1977) led to the proliferation of research into ways of preserving crops without formation of fermentation acids. The trend was not universally accepted at first, however, and Owen (1971) grouped carbon dioxide, sulphur dioxide and formaldehyde as sterilants which would have no potential for use in farming.

The characteristics of formalin treated silages are particularly hard to define, and variations in response to application rate have been observed by many workers (Wilkins et al, 1973; Valentine and Radcliffe, 1975; Wilkins, 1975; Castle, Retter and Watson, 1977; Hinks and Henderson, 1977).

The potential antimicrobial activity of formaldehyde has been demonstrated by Wilkins et al (1973), using formalin in a 1:2 serial dilution-type experiment with cultures of 33 microbial strains likely to be important in green crop fermentation and

in aerobic deterioration of silage after opening the silo, as well as some 'general laboratory' strains. Each combination of microbial species and formalin concentration was tested at pH 4.0, pH 5.0 and pH 6.0 and the minimum inhibitory concentrations were identified for each strain. The highest minimum inhibitory concentration measured was 8 mM, corresponding to application of formalin at the rate of 0.57 $l\ t^{-1}$ to a crop with a dry matter of 200 g kg^{-1} fresh weight. Their incubation conditions (30°C for 24 h) might be considered to be insufficiently long to detect very slow microbial growth which would become significant over an ensilage period of 50 d or more.

The same workers continued their investigations using laboratory scale silos. Cellulose wadding, a sugar/protein/salt broth and a buffer were placed in a glass tube and adjusted to dry matter and soluble carbohydrate levels of 200 g kg^{-1} and 160 g kg^{-1} DM respectively. This model silage was inoculated with seven representative strains of silage bacteria and treated with formalin at seven different application levels ranging from 0 to 9.1 $l\ t^{-1}$. The validity of the technique was demonstrated by the production of a typical 'lactate' silage with lactate and ammonia N levels of 79 and 0.7 g kg^{-1} DM respectively, in the untreated tubes. Formalin at 0.57 $l\ t^{-1}$ initially retarded the formation of fermentation acids and concomitant reduction in pH, but after a 64 d ensilage period no differences could be detected between these silages and the untreated controls. At formalin application rates above 2.3 $l\ t^{-1}$, total acid production was consistently below 20 g kg^{-1} DM, thus meeting the criteria proposed by Wilson and Wilkins (1974) for a non-fermented

silage. Ammonia N levels were low and pH did not fall below 5.20.

The final stage of this work by Wilkins and his co-authors involved test-tube scale analysis of the effects of the application of formalin to harvested forage crops. Ryegrass (173 g DM kg^{-1}, 175 g water-soluble carbohydrate kg^{-1} DM) was harvested and precision chopped before being treated with one of five formalin applications (0, 2.3, 4.5, 9.1 and 18.3 l t^{-1}). Again a representative lactate silage was produced when no formalin was applied - encouraging confidence in the applicability of the technique. All replicates of the lowest application rate yielded a silage in which typical clostridial fermentation had occurred. Butyrate levels were high indicating saccharolytic clostridial activity while the high ammonia N levels (360 g kg^{-1} total N) indicated extensive deamination by clostridia (McDonald et al, 1973). Formalin applied at 4.5 l t^{-1} resulted in a fermentation pattern not unlike that observed in the control silage, although no butyrate could be detected, whereas the two highest formalin treatments severely restricted fermentation, very low levels of fermentation acid and ammonia N being observed.

When ensiling two-tonne samples of ryegrass in butyl rubber silos for feeding experiments, Beever et al (1977) treated one batch with formalin at 6.4 l t^{-1} nominal application rate. After a 90 d ensiling period this formalin-treated silage was found to have similar total N and amino acid N levels to an untreated control ryegrass silage, whereas water-soluble carbohydrate levels were considerably elevated at the expense of the lactate and acetate concentrations. Fermentation was not,

as would have been expected from the work of Wilkins et al (1973), completely stopped, perhaps reflecting greater formaldehyde losses in the larger scale operation.

On the basis that low formalin applications might induce secondary clostridial fermentation Castle et al (1977), when producing silages for feeding to dairy cows, employed a formalin (66%) sulphuric acid (15%) mixture at 2.0 l t^{-1} and 4.4 l t^{-1} ("Sylade", produced by ICI). Neither application stopped fermentation although acid production was "assessed" to have been suppressed. No untreated control silage was made. The acid, however, had no effect in reducing the potential for clostridial fermentation and high butyrate and ammonia N levels were observed with both treatments.

In a more satisfactorily designed experiment Hinks and Henderson (1977) produced a range of silages for feeding to beef cattle. Formalin was applied at 2.3 l t^{-1} and 1.1 l t^{-1} with equal amounts of "Add-F" (85% w/w formic acid, BP Nutrition (UK) Ltd). Fermentation restriction was again observed (fermentation acid and ammonia N levels depressed, water-soluble carbohydrate and protein N levels increased compared with the untreated control), although obviously the interaction between the acid and the aldehyde makes causal identification difficult.

Some atypical results were obtained by Valentine and Radcliffe (1975) working in Australia. Here silages made from a grass/clover mixture treated with 4.6 or 9.2 l formalin t^{-1} at ensiling were compared with an untreated control. Compared with the control, high formalin application reduced ammonia N and total fermentation acid levels, but lactate concentration was increased.

At the lower formalin level the fermentation pattern was similar to that of the control silage except that lactate concentration was increased even more.

Variable dose:response observations may be attributable to very erratic application rates in the field due to the volatility of formaldehyde. In view of these deficiencies Wilson and Wilkins (1978) tested the effects of paraformaldehyde (a solid polymer of formaldehyde) on silage fermentation. Crops (ryegrass and lucerne) were treated with formalin, paraformaldehyde prill or paraformaldehyde powder before ensiling (all treatments being applied at 0, 1, 2 and 4 kg HCHO t^{-1} (6.25 l formalin t^{-1} = 2.5 kg HCHO t^{-1})). Paraformaldehyde, whether added as a prill or as a powder, acted in a similar manner to formalin, although at high application levels fermentation restriction was not quite as effective. A much higher percentage of the formaldehyde added as paraformaldehyde was recoverable (i e was present unbound to protein) compared with that added as a 38% w/v aqueous solution.

2.1.3.4.2 Mineral acids

The original aim of applying mineral acids to grass before ensiling was the attainment of a pH of 4.0 or less within the ensiled mass to prevent the fermentation of lactate to butyrate by clostridia (Virtanen, 1933). Initial experiments by Virtanen indicated that considerably smaller amounts of mineral acids (hydrochloric acid and, to a lesser extent, sulphuric acid) than lactic or phosphoric acids were required to achieve a given pH drop with a given crop type. Virtanen advocated the use of hydrochloric acid at the rate of 5 kg t^{-1} ensiled (equivalent

to 70 l, 2 M hydrochloric acid per tonne ensiled) and observed that at this application level butyrate production was completely prevented and that other normal fermentation processes were severely restricted. Depression of dry matter intake, acidosis and hypomagnesaemia were not observed unless sufficient acid was added to reduce the pH of the silage to 2.5 or sulphuric acid alone was used as the acidifying agent. After these promising beginnings the "AIV" process (after A I Virtanen, the originator), although widely used in Scandinavia, declined in popularity elsewhere. This demise was due principally to the difficulty in handling the corrosive acids (Owen, 1971; McDonald et al, 1973; McDonald and Whittenbury, 1973; Ekern et al, 1975; Wilkinson et al, 1976) and also to the fact that Virtanen's original claims of unaffected dry matter intake and animal health could not always be substantiated (Ekern et al, 1975).

Mineral acids may act to restrict selectively, rather than prevent, fermentation (see Section 2.1.3.5 below) but this often results from application at levels far below those recommended by Virtanen (Carpintero et al, 1979).

Mineral acids appear to function entirely by the effects of pH reduction (increased hydrogen ion concentration) on silage microorganisms (Davis et al, 1973). In an extensive experiment, in which compounds were screened for anti-microbial activity, Woolford (1978) was unable to demonstrate any specific anti-microbial effects of hydrochloric or sulphuric acids. Use of the AIV process persisted in Scandinavia, its success due partly to the development of better techniques for handling the acids (McDonald and Whittenbury, 1973; Ekern et al, 1975) and partly

to the increased confidence gained by continued accident-free use of a supposedly dangerous liquid (Drysdale, 1979). Even here, however, formic acid has now become the most widely used silage additive (Crawshaw, 1977).

Inorganic acids are found in several commercially available additives (Crawshaw, 1977) although the relative importance of these acids to the claimed effect of the additive appears to vary widely. For all, the application rate of acid is so low, when the additive is administered at the manufacturer's recommended dose, that no appreciable response could be expected (see Virtanen, 1933).

2.1.3.4.3 Formic acid

Formic acid may be applied merely to prevent clostridial fermentation by ensuring a rapid fall in pH, but its potent antimicrobial properties mean that when applied in sufficiently high concentration it functions in a class 2 mode (Carpintero et al, 1969; Davis et al, 1973; Carpintero et al, 1979).

The work of Carpintero et al (1969) involved treatment of lucerne with formic acid applied at the rate of 10 kg t^{-1}. The experiments were conducted using 1.2 kg capacity laboratory silos. No evidence of microbial activity (i e no fermentation acid or ammonia N production) was observed. With no microbial activity the water-soluble carbohydrate content of the silage after a 34 d storage period was actually higher than that of the inserted material, probably due to partial hydrolysis of hemicellulose. Such complete inhibition of clostridia and, in particular, Lactobaccili has not been recorded elsewhere, but numerous reports of restriction of fermentation have been presented.

Henderson and McDonald (1971) noted that although the use of formic acid had been advocated as early as 1926, its exploitation on a commercial scale had required the development of an applicator, mounted on the forage harvester, suitable for use with relatively undiluted acid. When these workers treated a grass mixture (timothy/fescue/perennial ryegrass) with formic acid at 2.2 or 3.4 kg t^{-1}, suppression of natural fermentation was evidenced by lower lactate and ammonia N, and higher protein N, levels than were observed in untreated controls. When the higher application rate was used, the ethanol content of the silage was higher than that of the control, indicating the differential action of formic acid on lactic acid bacteria and yeasts, allowing the latter to proliferate.

Bergen (1975) noted that formic acid application to direct cut ryegrass reduced dry matter losses, and the production of fermentation acids and ammonia during the ensilage period. Similar fermentation effects were noted when maize (Zea mais), treated with formic acid, was ensiled, but whereas improved animal performance was noted for treated vs untreated ryegrass silages, such benefits were not reported for treated maize silages.

Again, in 1976, Henderson and McDonald reported on the effects of formic acid as a pre-ensiling additive for ryegrass. By ensiling forage at different stages of maturity, different dry matters and with varying levels of formic acid, they were able to produce equations relating these parameters to the water-soluble carbohydrate, pH, volatile N and energy contents of the resulting silages. They concluded that with young grass,

formic acid application would encourage a natural lactate fermentation. Very high rates, or some pre-ensiling wilting, would result in a degree of restriction of fermentation. With increasing crop maturity and dry matter, the higher rates of formic acid produced silages where the restriction of fermentation was increasingly pronounced.

Barry et al (1978) ensiled young, unwilted, lucerne, treated with formic acid (85% w/w solution) at 1.5, 3.0 and 6.0 l t^{-1}. The level of water-soluble carbohydrate in each of these silages was higher than that in an untreated control and the acid also reduced the levels of fermentation products which could be attributed to clostridial activity. Proteolysis was also attenuated, but not to the same degree as had been achieved when formalin was applied before ensiling.

In test-tube scale experiments Carpintero et al (1979) demonstrated that formic acid added to a ryegrass/clover mixture at rates of 3.5 and 6.6 l t^{-1} had a significantly greater suppressive effect on the production of fermentation acids than had sulphuric acid. The extent of proteolysis and subsequent deamination was also reduced with increasing rate of formic acid application. However, even the highest application rate, which immediately reduced the pH of the grass to 3.50, did not prevent proteolysis : protein N levels were 819, 550 and 462 g kg^{-1} total N for the grass/clover mixture after harvest, and the ensiled material after 4 d and 50 d respectively. On the basis of their findings Carpintero et al (1979) produced regression equations relating application rate of formic acid to the protein N and ammonia N concentrations in the resulting silage.

Formic acid, marketed throughout Europe as an 85% aqueous solution ("Add-F, BP Nutrition (UK) Ltd) is the most commonly used additive for silage making (Crawshaw, 1977). Where its use is well established, its caustic nature has been overcome by the use of appropriately designed applicators and by observation of simple safety regulations (Drysdale, 1979). Continued reticence, however, prompted BP to develop a complex acid salt of formic acid. Ammonium tetraformate was selected as the most appropriate due to its reduced volatility and much reduced corrosive properties. In laboratory and field experiments, similar acid conditions to those achieved by the application of Add-F were obtained with concomitant reduction in the production of lactate, butyrate and ammonia (Drysdale, 1979). The manufacturers felt that the acid salt would only find favour amongst silage producers who were not successfully using Add-F.

2.1.3.4.4 Other fermentation inhibitors

Systematic screening of potential silage additives for antimicrobial activity has only recently been undertaken. Woolford (1979) carried out extensive experiments in which 33 pure cultures of silage-associated microbes were subjected to treatment by a wide range of additives, both potential and current. Woolford found that acrylic acid (tested because of its availability as a bulk chemical) had ten times the antibacterial activity of formic acid and was also active against yeasts and moulds, associated with aerobic spoilage, at pH below 5.0. Woolford suggested that acrylic acid applied at a rate of 0.5 l t^{-1} would stop all fermentation if the pH of the ensiled mass was reduced to 5.0. In subsequent experiments at the Grassland Research Institute

in Hurley, Wilson, Woolford, Cook and Wilkinson (1979) demonstrated that acrylic acid applied directly, or as its less corrosive sodium salt, was more effective than formic acid in preventing proteolysis and the formation of fermentation acids, while at the same institute Wilkinson, Cook and Wilson (1979) demonstrated similar performance when acrylate and formic acid treated silages were fed to growing Hereford x Friesian steers.

Antibiotics, and other components known to exhibit bacteriostatic properties (e g sodium metabisulphite, a source of sterilant SO_2, and carbon bisulphide) have been identified as potential additives (Virtanen, 1933; McDonald et al, 1973; McDonald and Whittenbury, 1973; Wilkins, 1975) but their use in commercial additives is severely restricted and manufacturer's recommended application rates are so low that any appreciable effects are unlikely (Owen, 1971; Crawshaw, 1977; Minister, 1978a).

2.1.3.5 Class (3) additives - selective stimulation of Lactobacilli

2.1.3.5.1 Formaldehyde

Formaldehyde is almost exclusively regarded as an inhibitor of all fermentation activity when applied to a forage crop at ensiling (McDonald et al, 1973; McDonald and Whittenbury, 1973). However, Valentine and Radcliffe (1975) reported selective Lactobacilli stimulation (evidenced by increased lactate production over an untreated control silage) when formalin was applied at 12 l t^{-1} and 6 l t^{-1} at ensiling. Such observations have not been substantiated by any other workers.

2.1.3.5.2 Formic acid

Although now considered to be an important antimicrobial additive, formic acid was initially considered to function by

providing acid conditions unsuitable for clostridial activity and thus selectively stimulating lactic acid bacteria (Owen, 1971; Henderson and McDonald, 1976). Subsequent observations of increased lactate production following low levels of formic acid application have been made (Carpintero et al, 1979). Henderson and McDonald (1971) observed elevated ethanol levels (compared with an untreated control silage) when formic acid was applied at 2.2 kg t^{-1}. This, however, indicated reduced formic activity against yeasts rather than against lactic acid bacteria.

2.1.3.5.3 Microbial inoculation

The most obvious method for ensuring the presence of active lactic acid bacteria within the ensiled mass is to provide an exogenous supplement of appropriate bacterial strains at ensiling. The many variables which can affect the response to inoculation with lactic acid bacteria (e g bacterial numbers and types present in the inocula and on the forage at time of harvest, fermentable water-soluble carbohydrate supply in the crop and crop dry matter) mean that any group of collated data is unlikely to demonstrate many significant trends (Owen, 1971) although Watson and Nash (1960) decided that the bulk of the evidence indicated that inoculation with lactic acid bacteria was not beneficial.

Carpintero et al (1979) used 2 ml (total count 2.5×10^4 organisms) of a mixed Lactobacillus plantarum, L. mesenteroides and Streptoccocus faecalis culture along with 0.69 g of glucose when inoculating 80 g samples of ryegrass before insertion in test-tube silos. This treatment produced a silage with higher lactate content (162 g kg^{-1} DM) than either formic or mineral acid applications and the rapidly induced fall in pH restricted

proteolysis. The use of a water-soluble carbohydrate supplement to ensure proliferation of the inoculated microbes is common in such experiments.

McDonald et al (1964) used a mixed inoculum containing eight different strains of homofermentative lactic acid bacteria selected for their ability to use the water-soluble carbohydrate of grass as a substrate and for their tolerance of a wide range of temperatures. Inocula were applied with and without supplementary water-soluble carbohydrate (molasses). Little beneficial effect was evident, when compared with untreated control ryegrass silage. However, when Cocksfoot grass, with a low water-soluble carbohydrate content, was ensiled, a beneficial response to inoculation with lactic acid bacteria particularly when supplemented with water-soluble carbohydrate, was observed. Compared with the untreated control, lactate levels were elevated, pH and butyrate were depressed.

Interesting work in Japan (Tasaki, Shibata and Kikuchi, 1970) recognised that inhibition of clostridia by the application of antibiotics or by inoculation with Lactobacilli were incompatible since the antibiotics would have "anti-Lactobacilli" effects. The development of antibiotic-resistant strains of L. plantarum was achieved and silages were experimentally inoculated with a mixture of microbes and antibiotics (streptomycin and bacitracin). Successful silages were produced with low butyrate and high lactate levels. However, no beneficial effects, compared with inoculation with Lactobacilli alone were demonstrated. The authors also demonstrated that the antibiotic zinc bacitracin, when applied at 0.005 or 0.010 kg t^{-1} to a 20% dry matter ryegrass

at ensiling, resulted in considerable clostridial inhibition without affecting the growth of lactic acid bacteria.

Important differences between the numbers of lactic acid bacteria detected in silages made from grass harvested by hand and chopped and ensiled in the laboratory, and in silages made from grass harvested and precision chopped by machine, have been observed. Bacteria numbers, and hence lactate levels, have been higher under "field" conditions. The importance of The forage harvester, coated with forage sap, as an inoculum source, has been investigated by Woolford and Hall (1974). They confirmed the low lactic acid bacteria levels associated with laboratory chopped silage, but demonstrated that when a sterilised bowl chopper which caused significantly more laceration than the usual laboratory guillotine was used, the fermentation acid patterns produced were identical to those obtained with material chopped through a forage harvester. Thus substrate availa bility rather than initial microbial population appeared to be the principal determinant of the proliferation of lactic acid bacteria.

2.1.3.6 Wilting

Increasing the dry matter in silage, by introducing a period of field drying between the cutting and ensiling of the crop, is a feature of many commercial silage-making systems (MAFF, 1977). Some of the reasons for the popularity of wilting are associated with the logistics of farm-scale operations rather than any considerations of the effects that increased dry matter content might have on the fermentation process.

Production of liquid effluent which, at 150 - 300 l t^{-1} for herbage with a dry matter content of 150 g kg^{-1}, is copious for unwilted silage, is reduced to insignificant levels if the crop is wilted to a dry matter of 250 g kg^{-1} (MAFF, 1977; Marsh, 1979). As well as being a potential source of loss of ensiled nutrients, the effluent from silage has a high "biological oxygen demand" and is a pollutant whose entry into watercourses must be avoided (MAFF, 1977). Wilting therefore removes, or reduces, the need for facilities to allow the short term storage and subsequent disposal of large volumes of this material.

By reducing the weight of water which has to be passed through the forage harvester, and then removed from the field with the forage, wilting is also likely to improve the work rate (t DM h^{-1}) of silage making (MAFF, 1977). However an overall benefit will only accrue if these advantages are not offset by the time and energy requirements of any additional tedding operations which may be required to achieve the desired level of dry matter in the crop (MAFF, 1977).

It has also been recognised that this particular method of manipulating the natural ensilage process has potential disadvantages. The most obvious is the technique's reliance on clement weather conditions to allow rapid drying of the swaths of cut forage (Ekern et al, 1975; MAFF, 1977; Marsh, 1979). Any tedding or windrowing operations which are required are likely to increase the loss of dry matter which occurs between the field and the silo (MAFF, 1977). By increasing the length of time between the cutting and ensiling of the crop, the potential for aerobic degradation of plant nutrients is increased (McDonald

and Edwards, 1976). Finally, the increased mechanical resilience of the partially dried forage means that adequate consolidation of the ensiled mass, to ensure that anaerobic conditions persist, is more difficult than in unwilted material (MAFF, 1977).

2.1.3.6.1 Effects of wilting on the fermentation process

During the field drying period aerobic respiration, catalysed by plant enzymes, occurs (McDonald and Edwards, 1976). Sugars, malate, citrate and succinate are oxidised and continued activity of plant proteases results in an increase in the levels of soluble or non-protein N (McDonald and Edwards, 1976). Thus Morgan et al (1980b) demonstrated that the non-protein N content of grass ensiled after a wilting period of 52 h was appreciably higher than that of similar grass ensiled directly after cutting (296.9 and 178.6 g kg^{-1} total N respectively).

It is considered that wilting has a generally suppressive influence on the fermentative activities of the silage micro-organisms (Henderson and McDonald, 1976; McDonald and Edwards, 1976; MAFF, 1977; Marsh, 1979; Thomas et al, 1980). Increasing the dry matter content to 250 - 280 g kg^{-1} ensures that clostridial activity is unlikely (McDonald and Edwards, 1976; MAFF, 1977; Thomas et al, 1980). The activity of this group of bacteria is arrested at a higher pH in wilted silage because of the complementary effect of increased osmotic pressure. Morgan et al (1980b) observed that ammonia N and butyrate levels, both indices of clostridial activity, were lower in wilted (359 g DM kg^{-1}) than in unwilted (169 g DM kg^{-1}) ryegrass silages. The values were 80.3 g ammonia N kg^{-1} total N, 1.1 g butyrate kg^{-1} DM and 108.9 g ammonia N kg^{-1} total N, 4.7 g butyrate kg^{-1} DM for the

wilted and unwilted silages respectively. Such restriction of clostridial activity can be important, and Henderson and McDonald (1976) predicted that the unstable, poorly preserved material which resulted from ensiling ryegrass at the pre-ear emergence stage when moisture and protein levels were high but soluble carbohydrates were low, could be avoided if the dry matter of the grass was increased to 250 - 350 g kg^{-1} prior to ensiling.

The activities of lactic acid bacteria are attenuated rather than abolished in wilted silages, and some growth of this group of bacteria continues even in silages wilted to a dry matter content of 500 g kg^{-1} (McDonald and Edwards, 1976). The lactate contents of the wilted and unwilted silages described above (Morgan et al, 1980b) were 34 and 165 g kg^{-1} DM respectively. Total fermentation acid levels were also lower for the wilted than for the unwilted silage (46.2 and 192.8 g kg^{-1} DM respectively). This restriction in the formation of acids has also been noted by Marsh (1979), and a concomitant increase in the proportion of plant water-soluble carbohydrates which remain in the silage, is frequently observed (Henderson and McDonald, 1976; McDonald and Edwards, 1976; MAFF, 1977; Marsh, 1979; Morgan et al, 1980b). In the silages made by Morgan et al (1980b), the water-soluble carbohydrate concentration in the wilted sample was, at 185 g kg^{-1} DM, 11% higher than in the grass as ensiled, the increase possibly being explained by the partial hydrolysis of plant heteropolysaccharides (Carpintero et al, 1969; McDonald, 1980). In contrast, the unwilted silage had a low level of residual water-soluble carbohydrates (11 g kg^{-1} DM).

McDonald and Edwards (1976) stated that reduced breakdown of protein might accompany the reduction in fermentation of carbohydrate in wilted silages and thus result in a more balanced provision of energy and nitrogenous nutrients for ruminants. However Marsh (1979) contended that the effects of wilting on protein breakdown in the silo were minimal. Typical silage compositions presented by McDonald and Edwards (1976) indicated only slight differences between unwilted 'lactate' silage (235 g protein N and 78 g ammonia N kg^{-1} total N) and wilted silage (289 g protein N and 83 g ammonia N kg^{-1} total N). Similarly, Morgan et al (1980b) observed protein N levels of 286.0 and 285.7 g kg^{-1} total N for unwilted and wilted ryegrass silages respectively.

2.2 MEASUREMENT OF THE RATE OF FLOW OF DIGESTA

2.2.1 Introduction

Feeding schemes for ruminant animals, based on the provision of known amounts of metabolisable nutrients, have been proposed in the United Kingdom for energy (Agricultural Research Council, 1965; 1980) and for protein (ARC, 1980). The metabolisable energy (ME) system has gained wide acceptance and is used for ration formulation and the prediction of animal performance by the official advisory services (MAFF, 1976). For protein, there is a lack of accurate and precise information on many of the factorial equations designed to relate dietary intake to intestinal supply.

In ruminant animals, the early intervention of microorganisms in the digestive process complicates the assessment of a diet's nutritive value. The facts that: a) not all components of the faeces are of dietary origin and b) non-appearance of dietary components in faeces is not synonymous with absorption from the digestive tract, mean that apparent digestibility is an inadequate measure of the supply of absorbable nutrients to the small intestine (Faichney, 1975b; Sutton and Oldham, 1977; Thomas, 1978). This, and consequently the apparent digestion of those nutrients in the gut anterior to the first part of the small intestine, can be measured in surgically modified animals. Once access has been gained to the lumen of the gut, the rate of flow of digesta may be measured. This information, when combined with a knowledge of the concentrations of nutrients in the digesta, allows calculation of the amounts of nutrients available for absorption and subsequent metabolism (Sutton and

Oldham, 1977; Thomas, 1978).

The rate of flow of digesta may be estimated using either of two groups of techniques (Corse and Sutton, 1971; Macrae and Ulyatt, 1972; Faichney, 1975b; Sutton and Oldham, 1977; Beever, Kellaway, Thomson, Macrae, Evans and Wallace, 1978). It may be measured directly, with the gut exteriorised for a short distance before being returned within the animal's body. This technique is known as re-entrant cannulation (Phillipson, 1952). Alternatively, the rate of flow of digesta may be determined indirectly by reference to the dilution, within the digesta, of a known amount of an indigestible marker substance (Kotb and Luckey, 1972; Faichney, 1975b).

2.2.2 Direct measurement of the rate of flow of digesta

Re-entrant cannulation of the ruminant small intestine was developed at Cambridge University in the late 1940's and early 1950's (Phillipson, 1952). The technique, and subsequent modifications of it, have provided the main means by which the flow of digesta and nutrients to, and the absorption of digestion products from, that organ have been measured in subsequent experiments (Lazenby, 1978). The surgical procedures employed may involve transection of the small intestine at the chosen site with cannulas inserted into, or near to, the cut ends of the gut (Ash, 1962). The cannulas are brought to the surface through the abdominal wall and connected externally with a short piece of tubing. More recent surgical developments have yielded preparations which achieve the same result, in terms of exteriorised digesta flow, but which avoid the necessity to transect the intestine (Egan and Ivan, 1979; Ivan and Johnstone, 1979).

Re-entrant cannulas may be inserted at any point in the small intestine but obviously when measurements of the supply of rumen products to the absorptive regions of the tract are to be made, the point of sampling should be at the start of that section of the intestine (Sutton and Oldham, 1977; Thomas, 1978). In sheep, the cannula can readily be placed in the proximal ascending duodenum, anterior to the common bile duct (Brown, Armstrong and Macrae, 1968). Indeed, the technique of Ash (1962) involves the placement of the proximal outflow cannula in the abomasal antrum, with the distal return cannula in the first part of the duodenum. Surgical difficulties, however, have meant that most work with mature dairy cows has involved cannulas positioned posterior to the bile and pancreatic ducts (Thomas, 1978). Exceptionally, re-entrant cannulation of the sheep may also be performed at this, less desirable, position (Wenham and Wyburn, 1978), allowing bile and pancreatic secretions to make indeterminate contributions to the composition of the digesta samples ultimately acquired (Sutton and Oldham,1977; Thomas,1978).

The principles of the use of a re-entrant preparation to measure the rate of flow of digesta in a cannulated animal are simple. Over a recorded time, all digesta issuing from the proximal, outflow, cannula is collected. Periodically, the quantity of digesta produced is measured and a sub-sample is taken for subsequent analysis. The remaining digesta is then returned through the inflow cannula (Phillipson, 1952; Harris and Phillipson, 1962; Corse and Sutton, 1971; Sutton and Oldham, 1977). Total flow

of digesta is therefore measured and, combined with the results of the compositional determinations, allows the calculation of the rate of flow of various nutrients to the region of the tract posterior to the site of cannulation.

In practice, these simple principles are invariably complicated. Firstly, consideration must be given to the return of digesta through the distal cannula. In some cases no replacement is provided for the sample of digesta which has been removed (Axford, Evans and Offer, 1971; Van Bruchem and Van't Klooster, 1980) or no indication is given as to whether or not this refinement has been employed (Gill, Ulyatt and Barry, 1979). Alternatively, the volume of digesta returned may be restored to that which was initially collected, by the addition of the appropriate amount of 'donor' digesta previously collected from the same, or a similar, cannulated animal (Hutton, Bailey and Annison, 1971; Macrae and Ulyatt, 1972; Oldham and Ling, 1977). With or without the addition of donor material, it will be appreciated that digesta must be returned frequently if its flow through the intestine, posterior to the re-entrant cannula, is not to be excessively episodic. Routinely, a collection period of 2 - 3 h will elapse before a sample is taken and the digesta returned (Beever et al, 1971; Corse and Sutton, 1971; Macrae and Ulyatt, 1972). When performed manually, such collection procedures are obviously time consuming and laborious (Axford et al, 1971) and therefore tend to be restricted to relatively short duration. Quoted daily rates of flow of digesta are usually the result of measurements performed over a single 24 h period (Beever et al, 1971; Macrae and Ulyatt, 1972; Beever et al, 1977; Chamberlain and Thomas, 1979) and periods as short as 12 h have been used (Corse and Sutton, 1971).

When short-term measurements of total flow are performed, an indigestible non-absorbable marker may be introduced to the tract at a point anterior to the point of digesta collection. The recovery of the marker, and hence the 'completeness' of the digesta collection, may then be determined (Sutton and Oldham, 1977). Despite its inadequacies for more complex marking of digesta (see Section 2.2.3.2.1 below), chromium sesquioxide (Cr_2O_3) fulfils the requirements for this task (Kotb and Luckey, 1972; Faichney, 1975b). The marker is generally administered intra-ruminally, either as Cr_2O_3-impregnated paper (Beever et al, 1977; Beever et al, 1971; Corse and Sutton, 1971; Macrae and Ulyatt, 1972), or as Cr_2O_3 contained within a gelatine capsule (Chamberlain and Thomas, 1979). Other digesta markers have been used for the same purpose. Corse and Sutton (1971) used polyethylene glycol (PEG) in an experiment where Cr_2O_3 was also used. The percentage recovery of PEG, at the duodenum, was appreciably lower than that for Cr_2O_3, and these workers concluded that PEG was being absorbed or metabolised. This non-ideal behaviour of PEG is now well documented (Kotb and Luckey, 1972; Faichney, 1975b; see Section below). Macrae and Ulyatt (1972), however, reported that correction of observed flow of digesta liquid to 100% recovery of either PEG or Cr_2O_3 yielded comparable results.

When such experiments are performed, the recovery of Cr_2O_3 is incomplete and the marker content of the digesta collected over a 24 h period is less than the concomitant administration of the same marker into the rumen (Axford et al, 1971; Macrae and Ulyatt, 1972; Oldham and Ling, 1977; Sutton and Oldham, 1977). Since the recoverability of the Cr_2O_3 itself is not disputed, the

conventional interpretation of such results has been that the collection procedures themselves cause, at least in the short term, a diminution in the flow of digesta (Axford et al, 1971; Macrae and Ulyatt, 1972; Sutton and Oldham, 1977). Thus, most experiments in which flow rate is ostensibly measured by total collection of digesta via a re-entrant cannula, actually collect slightly less than all of the digesta which pass the sampling point during the collection period. The extent of this shortfall is then estimated from the recovery of an indigestible marker, administered at a known rate prior to, and during, the collection period. Observations of depressed rates of flow during short term collections are not, however, universal. In a series of experiments using seven cross-bred wethers fitted with re-entrant duodenal cannulas, Oldham and Ling (1977) failed to demonstrate a statistically significant difference in the flow of dry matter over three consecutive 24 h collection periods. The rate of flow calculated from the first 24 h period was also indistinguishable from that measured over 72 h. The fact that Oldham and Ling were careful to ensure that the experimental animals were well acclimatised to the collection procedures, may have contributed to their success.

The laborious, and perhaps disruptive, nature of manual collection procedures, coupled with a desire to perform collections of longer duration, has resulted in the development of completely automated apparatus for collecting and sampling digesta (Axford et al, 1971). The apparatus developed by Axford and his co-workers at the University College of North Wales, Bangor, illustrates the principle of all such automated techniques. Digesta flows

from the outflow cannula into a collection vessel. When the level of material in this beaker reaches a threshold level, a digesta return/digesta sampling sequence is initiated with material pumped back into the inflow cannula and, briefly, into a second collection vessel where a representative sample for subsequent analysis is thus gradually accumulated. In their experiments, Axford et al (1971) found that the experimental animals (sheep with Ash-type re-entrant cannulas in the proximal duodenum) became completely acclimatised to the collection procedures, and uninterrupted collection periods of up to 16 weeks were possible. Recovery of orally administered Cr_2O_3 was also quantitative (103.5 $\pm$ 2.2%, x $\pm$ SEM, n = 14) and thus uncorrected collections of digesta were feasible. It should be noted that the observations of complete recovery of the marker were made with a single cannulated wether.

2.2.2.1 Practical applications of direct measurement of flow rate

Despite these handicaps and practical complexities, which detract from what is, in principle, a simple technique, re-entrant cannulation of the ruminant small intestine has seen widespread use in experiments in which rates of flow, nutrient supply and nutrient absorption have been studied (Lazenby, 1978). For example, the technique was used by a group of workers at the University of Newcastle upon Tyne in a series of experiments characterising the digestion of dried grass in sheep (Beever et al, 1971; Beever et al, 1972; Coelha da Silva et al, 1972). The animals used were mature Suffolk-cross wethers fitted with rumen and re-entrant duodenal and ileal cannulas. Manual collection of digesta, and correction of observed flow rates to 100% recovery of intra-ruminally administered Cr_2O_3 paper, was employed. The workers were able

to demonstrate changes in digestion patterns attributable to grinding and pelleting dried ryegrass (Lolium perenne cv. S24). Overall, organic matter digestibility was depressed, but this whole-animal effect masked the fact that the proportion of organic matter disappearing in the tract anterior to the duodenal cannula was markedly reduced whereas disappearance from the small intestine (as measured by rates of flow of organic matter at the duodenum and ileum) was proportionately increased (Beever et al, 1972). Similar trends were observed for the disappearance of apparently digested N in the gastrointestinal tract (Coelha da Silva et al, 1972). In the case of this nutrient, however, an apparent gain was recorded between the mouth and the proximal duodenum, the gain being greater when the material had been pelleted. Once more, pelleting increased apparent absorption of the nutrient from the small intestine.

This technique has been used regularly at the Grassland Research Institute, Hurley. Beever et al (1977) used sheep fitted with re-entrant cannulas to compare the digestion of, and nutrient supply from, silages made with and without an additive containing formaldehyde. The supply of organic matter and total amino acids was increased by formalin treatment but digestion of organic matter and amino acid N in the small intestine was not.

Similarly, Thomas, Chamberlain, Kelly and Wait (1980), working at the Hannah Research Institute, Ayr, investigated the passage, to the duodenum, of nitrogenous nutrients of silages made from L. perenne cut at different times in the season and ensiled with formic acid. With a silage made from spring-cut grass, they demonstrated an increased supply of nitrogen to the duodenum when intake of total N was increased by feeding a barley supplement. Output

of total N in the faeces was not increased in proportion, thus indicating increased intestinal absorption of this nutrient.

2.2.3 Indirect measurement of digesta flow rate

2.2.3.1 Introduction

Dilution of a known amount of an indigestible marker substance in the digesta within the gastrointestinal tract provides a means by which the total quantity of digesta present can be estimated (Kotb and Luckey, 1972). Provided that the concentration of the marker can be determined in a representative sample of digesta, the dilution of the original marker can be calculated. If an indigestible marker substance is continuously infused into the gastrointestinal tract, and its dilution in the digesta is measured when its concentration has stabilised, flow rate at the point of sampling may also be calculated as infusion rate divided by marker concentration (Faichney, 1975b). This is the principle by which digesta flow rate may be measured indirectly. It has many attractions when compared with the direct, re-entrant, technique. However, as with that method, current experimental practice is considerably more complex than theory would indicate.

Administration of the chosen marker may be episodic (Morgan, Edwards and McDonald, 1980; Corse and Sutton, 1971). Here, the experimenters assume that slow release and thorough mixing of the marker in the tract anterior to the point of sampling will ensure a stable marker:digesta ratio in the digesta sample. Alternatively, the marker may be uniformly mixed in the food, either by virtue of being an endogenous component of the diet (Waller, Merchen, Hanson and Klopfenstein, 1980) or through careful incorporation of an exogenous substance (Goshtasbpour-Parsi, Ely,

Boling, Alderson and Amos, 1974; Beever, Kellaway, Thomson, Macrae, Evans and Wallace, 1978). A third approach is to administer a solution of an exogenous marker continuously at a point in the gut anterior to the cannula through which the digesta sample will be taken (Faichney, 1975b; Beever et al, 1978).

In principle, the technique will work with a single indigestible marker. However, digesta consists of two phases, solid and liquid (Faichney, 1975b; Waller et al, 1980). Two separate markers, one associating with each phase, may therefore be required (Faichney, 1975b; Waller et al, 1980; see Sections 2.2.3.2.1, 2.2.3.2.2).

Finally, the sample which is obtained from a simple T-piece cannula may not be representative of the digesta at that point in the tract. This problem can be overcome by employing the dual-phase marker technique (Faichney, 1975b) which uses the concentrations of two markers, in the digesta and in a centrifuged subsample, to reconstitute mathematically true digesta which would have been obtained if the original sample had not been biased (Faichney, 1975b, 1980; see Section 2.2.3.2.3; Appendix A).

If such an indirect system is to yield accurate estimates of flow rate, the choice of marker, marker administration, animal preparation and sampling technique must combine to allow the reliable acquisition of a representative sample of the digesta flowing past the point of sampling. The administered marker must be quantitatively recoverable and the marker, its administration, the surgery performed and the sampling technique must not have disrupted the animal's normal physiology.

2.2.3.2 Indigestible marker substances

The qualities required of a substance before it can be considered an effective nutritional marker were presented by Kotb and Luckey

in their extensive review (1972). These criteria appear to have been generally accepted by workers in the field of ruminant nutrition, and Macrae (1974) and Faichney (1975b) both used abbreviated versions of the earlier workers' list when citing the factors which they considered desirable in a marker. The original attributes required by Kotb and Luckey (1972) were that the substance should:

(i) be inert with no toxic, physiological or psychological effects,

(ii) be neither absorbed nor metabolised within the alimentary tract and therefore be recovered from either raw or processed food,

(iii) have no appreciable bulk,

(iv) mix intimately with the usual food and remain uniformly distributed in the digesta,

(v) have no influence on alimentary secretion, digestion, absorption, normal motility of the digestive tract or excretion,

(vi) have no influence on the microflora of the alimentary tract which is of significance to the host,

(vii) have qualities which allow ready, precise, quantitative measurements,

(viii) have physical-chemical properties which make it discernible throughout the digestive process.

For a substance to perform the specific role of a marker allowing the indirect measurement of digesta flow rate, these general criteria must all be met. It is only relatively recently that substances with these attributes have been used in studies

of ruminant nutrition (Macrae, 1974).

The earliest attempts to measure digesta flow rate, in man and in several non-ruminant animal species, employed inert particulates (glass beads, small seeds, gravel and metal fragments) as markers (Kotb and Luckey, 1972). Such techniques were unsatisfactory since the materials did not fulfil criterion (iv) above, their rates of flow being inversely related to their specific gravities, and the distributions within the digesta therefore not becoming uniform. Faichney (1975b) used slightly different wording when describing the particular attribute of markers that inert particulates do not fulfil. He stated that the marker must "be physically similar to, or intimately associated with, the material it is to mark". The material in question is, as Kotb and Luckey (1972) and Macrae (1974) stated, "digesta". It is obvious, however, that digesta contains material in two physical states, solid and liquid, and there are therefore two phases with which the marker must associate. If these components move at different rates through the tract, no single marker can be expected to remain intimately associated with, or uniformly distributed in, the composite material. This fact is now generally appreciated amongst workers studying digestive function in ruminants, and many have concluded that the dilution of a single marker cannot provide accurate assessment of digesta flow rate (Drennan, Holmes and Garrett, 1970; Faichney, 1972; Macrae, 1974; Faichney, 1975b; Faichney, 1980).

Thus, even the simplest marker for measuring the flow of digesta indirectly must consist of two components - a solid phase marker and a simultaneously administered liquid phase marker.

Having established that two markers with different properties are required, those which in general conform to the criteria of Kotb and Luckey (1972) may be critically evaluated.

2.2.3.2.1 Solid phase markers

Chromium sesquioxide

Chromium sesquioxide (Cr_2O_3) has probably been the most widely used inert marker in nutritional studies (Kotb and Luckey, 1972; Macrae, 1974). The traditional use to which it has been put, faecal marking for faecal output and digestibility estimations in human (Sharpe and Robinson, 1970), non-ruminant (Schürch, Lloyd and Crampton, 1950; Mueller, 1956) and in ruminant animals (Kotb and Luckey, 1972; Macrae, 1974), does not require that the marker should associate closely with any particular phase of the digesta. Criteria of inertness and non-absorbability must be met, and the material must, additionally, be fully recoverable in the faeces. That this is the case is not disputed (Corbett, Greenhalgh, McDonald and Florence, 1960; Macrae and Armstrong, 1969; Beever et al, 1978). Similarly, when Cr_2O_3 is used to correct short term, apparently complete, collections of digesta from animals fitted with re-entrant cannulas (Macrae and Ulyatt, 1972; Sutton and Oldham, 1977; see Section 2.2.2 above), all that is required is that the material be quantitatively recoverable over a collection period long enough to take into account diurnal variation in marker flow (Macrae and Armstrong, 1969; Macrae, 1974).

Chromium sesquioxide has also been used both alone and in conjunction with liquid phase markers, to determine the rate of flow of digesta to the small intestine of ruminants fitted with simple T-piece cannulas. The preponderance of evidence has suggested

that, when used in this manner, Cr_2O_3 does not yield accurate values (Drennan et al, 1970; Faichney, 1972; Macrae and Ulyatt, 1972; Sutton and Oldham, 1977; Beever et al, 1978; Crickenberger, Bergen, Fox and Gideon, 1979). Other workers, however, have maintained that, provided criteria on the representative nature of the digesta sample are met (see below), Cr_2O_3 dilution is a valid indicator of digesta flow rate (Corse and Sutton, 1971; Goshtasbpour-Parsi et al, 1974; Thomas, 1978; Merchen et al, 1980; Morgan et al, 1980).

Using three sheep fitted with re-entrant duodenal cannulas and three provided with simple T-piece cannulas at the same site, Corse and Sutton (1971) compared digesta flow rates determined by three different methods. When feeding two diets, which differed in their proportions of hay and concentrates but which were approximately isocaloric, these workers were unable to demonstrate significant differences in the rates of dry matter flow at the duodenum measured by conventional, Cr_2O_3 corrected, total digesta collection from animals fitted with re-entrant cannulas or indirect assessment based on the dilution of Cr_2O_3 in bulked 'spot' samples taken either from the proximal outflow of the re-entrant cannula or from the T-piece cannula. The Cr_2O_3 was administered, intraruminally, twice daily as impregnated paper. It was significant that Corse and Sutton (1971) did not show any real difference in calculated flow rate between the two spot sampling methods. That using the digesta acquired from the re-entrant cannula might be expected to yield more accurate values since the digesta sample might be presumed to be wholly representative of duodenal contents.

Goshtasbpour-Parsi et al (1974) used Cr_2O_3 as the solid phase component of a two-marker system when measuring flow rates to the omasum and abomasum of four wethers. The marker was incorporated into semi-purified diets, which were offered in equal amounts at twelve-hourly intervals. The solid phase was deemed to be material precipitated by centrifugation (20000 x g, 30 min) and the Cr_2O_3 content of this fraction allowed calculation of its flow rate.

In another experiment where Cr_2O_3 was used as the solid phase component of a two-marker system with polyethylene glycol (see below) as the liquid marker, Waller et al (1980) compared its performance with two endogenous particulate markers (indigestible neutral detergent fibre and indigestible acid detergent fibre, see below). Again, the particulate fraction was isolated by centrifugation, although here a less severe regime was employed (500 x g, 20 min). Despite the fact that estimates of the rates of flow of several components of digesta varied significantly depending on the marker used, with the highest flow rates being obtained with Cr_2O_3, the workers concluded that Cr_2O_3 remained a satisfactory particulate phase marker.

Morgan et al (1980) used Cr_2O_3 alone as a marker for measurement of the rates of flow of digesta in abomasally cannulated sheep. They administered the marker as Cr_2O_3 impregnated paper, and used a thorough sampling schedule (two-hourly intervals over a 24 h period) in an effort to obtain a representative bulked sample.

In a recent review, Thomas (1978) considered the relative merits of Cr_2O_3 and more complex methods employing two markers. While recognising that Cr_2O_3 had attributes which others considered

sufficient to render it unsuitable as a flow rate marker (principally its indeterminate association between the solid and liquid phases of digesta), Thomas asserted that it could yield valid results if the sample collected from the cannula was representative of the digesta flowing past it. He accepted that this was not the case when the cannula was placed in the abomasum, but considered that sampling from duodenal or ileal T-piece preparations was not selective.

The principal reason why Cr_2O_3, used alone, has been rejected by many workers has been that its association with either particulate or liquid fractions of digesta has not been intimate (Drennan et al, 1970; Faichney, 1972; Macrae and Ulyatt, 1972; Faichney, 1975b). Others have cited the variability of flow rates derived from the use of this substance (Macrae and Ulyatt, 1972; Beever et al, 1978; Crickenberger et al, 1979). Beever and his fellow workers (1978) provided a clear elucidation of the areas in which the dilution of Cr_2O_3 in a digesta sample acquired from a T-piece cannula is deficient as an estimator of flow rate. Their experiments compared the technique with a similar, indirect, procedure using tris (1,10-phenanthroline) ruthenium (II) chloride (Ru-P) and the chromium complex of ethylenediamine tetraacetic acid (Cr-EDTA) as solid and liquid phase markers respectively (see Section 2.2.3.2.3 below). By using this second, dual-phase, system the experimenters were able to ascertain the extent to which the composition of the sample acquired through the cannula differed from the digesta in the tract. They were able to do this because of the relationship which exists between the markers' infusion rates and their concentration in digesta. Once equilibrium has been established,

the concentration of each marker in the digesta within the tract, expressed as a proportion of its daily infusate must be the same (Faichney, 1975b; see Section 3.7; see Appendix A). At high rates of digesta flow, when both marker concentrations were, in absolute terms, low, the digesta samples were unrepresentatively weighted towards the liquid phase of digesta. With low flow rates and high marker concentrations, the solid phase of digesta, as marked by the Ru-P, was withdrawn from the tract in amounts greater than were warranted by its proportion in gut contents. If this sampling bias occurs, a single digesta marker cannot possibly give accurate estimates over a range of flow rates. Beever et al (1978) observed that, compared with rates estimated using Ru-P plus Cr-EDTA, Cr_2O_3 underestimated at low and overestimated at high the rates of flow of six individual nutrients. Given the sampling biases identified, these results would have been expected from a marker tending to associate more with the solid phase of digesta. While not commenting unequivocally on the absolute accuracy of their techniques, Beever et al (1978) considered that previous experiments, demonstrating good agreement between spot sampling techniques using the radio-labelled versions of their dual-phase markers (^{103}Ru-P plus ^{51}Cr-EDTA) and total collections from re-entrant cannulas (Macrae and Ulyatt, 1972), indicated that the Cr_2O_3-derived flow rates in their experiments were more likely to be inaccurate.

Tris (1,10 phenanthroline) ruthenium (II) chloride (Ru-P)

Ruthenium is a Group VIII transition element belonging to the relatively rare platinum metals sub-grouping (Mahan, 1966). It is a hard, grey, brittle metal which shares the generally inert nature of the platinum metals, but which does react readily

with oxygen and, at elevated temperatures, the halides. The oxide, halides and sulphide are the only ruthenium compounds that do not involve coordination or complexing of the metal atom (Mahan, 1966).

At low concentrations ($<10^{-11}$M), certain radioactive rare-earth, and other transition, elements exhibit what are known as radio-colloidal properties and tend to adhere strongly to particulate matter (Schweitzer and Jackson, 1952). This has been exploited in nutritional studies where radioisotopes of several of the rare-earth elements have been used as digesta and faecal markers (Kotb and Luckey, 1972).

Early work with ruthenium used the radioactive material, ^{106}Ru, a β-emitting isotope obtained along with significant quantities of ^{103}Ru after the fission of ^{235}U (Thompson, Weeks, Hollis, Ballou and Oakley, 1958). These workers investigated the distribution of ^{106}Ru in the digestive tract of rats killed at intervals from 35 min to 30 h after the administration of a solution of ^{106}Ru as the labelled chloride. They concluded that the radioactivity was associated with food residues in the tract and that the passage of the marker was not impeded by adsorption onto the intestinal wall.

The use of the phenanthroline complex of ruthenium as a marker of the particulate phase of digesta was proposed and developed by the Division of Animal Physiology of the Commonwealth Scientific and Industrial Research Organisation in Australia (CSIRO, 1969; Tan, Weston and Hogan, 1971). Tan et al (1971) demonstrated that ^{103}Ru-labelled tris (1,10 phenanthroline) ruthenium (II) chloride (^{103}Ru-P) was strongly adsorbed onto the particulate phase of

digesta over the pH range 3 - 8, that it had no overall effect (at concentrations $<10^{-5}$M) on volatile fatty acid and ammonia production by rumen microorganisms and that it was not absorbed, appreciably, from the alimentary tract. Additionally, the gamma emission from the radioactive ^{103}Ru allowed rapid, sensitive and quantitative determination of the marker's concentration in digesta samples (Macrae, 1974). As a radioactive marker, ^{103}Ru-P thus had superior attributes to alternative rare-earth isotopes such as ^{144}Ce, which is adsorbed onto glassware and has a relatively long half-life of 285 d (Kotb and Luckey, 1972; Macrae, 1974).

The use of material which presents an ionising radiation hazard is strictly controlled in the United Kingdom, and the small, but measurable, emission from ^{103}Ru-P (0.42 - 0.60 MeV gamma emission) means that its use is restricted to centres where the appropriate handling and disposal facilities are available (Macrae and Evans, 1974; Evans, Macrae and Wilson, 1977; Beever et al, 1978). With the development of analytical techniques allowing the determination of non-radioactive ^{101}Ru, use has been made of unlabelled tris (1,10 phenanthroline) ruthenium (II) chloride (Ru-P) as an alternative particulate phase marker (Macrae, 1974; Macrae and Evans, 1974; Evans et al, 1977; Beever et al, 1978).

Inert Ru-P was first prepared at the Hill Farming Research Organisation in Scotland (Macrae and Evans, 1974). The complex was prepared according to the procedure described by Tan et al (1971) for radiolabelled ^{103}Ru-P. Macrae and Evans (1974) reported that the ruthenium of the administered marker was fully recoverable in faeces (the average recovery for a series of three experiments was 99.5%) and that ruthenium adhered almost exclusively to the

particulate phase in digesta, over a range ofpH, and in faeces. The same workers reported more fully on their experimentation with Ru-P in a later publication (Evans et al, 1977). Inert ruthenium was measured by X-ray fluorescence spectrophotometric analysis, a technique which allowed simultaneous determination of the chromium component of the liquid phase marker which had been administered simultaneously (Cr-EDTA, see below). The lower limits of detection were 2.4 mg kg^{-1} and 1.8 mg kg^{-1} for ruthenium in solid and liquid samples respectively. The marker's adsorptive properties were studied at pH 3, pH 6.6 and pH 8 in vitro, when Ru-P was added to whole rumen digesta, and in vivo in an experiment in which Ru-P was continuously infused at two rates into the rumen of two sheep and faecal samples were taken periodically. In the in vitro studies, 92 - 96% of administered ruthenium was recovered in the residue resulting from centrifugation (3000 x g, 30 min), whereas 96% of the marker was associated with a similar particulate residue following dilution, homogenisation and centrifugation of the faecal samples taken in vivo. The workers were unable to explain the fundamental biochemical or biophysical processes involved in the adsorption of Ru-P, or ruthenium, onto the solid phase of digesta. When the dry matter component of the supernatant of faecal homogenates (which could not be clarified completely by centrifugation) was obtained by evaporation, it contained ruthenium at similar levels to those encountered in the original solid phase sample. This indicated that adsorption of ruthenium was independent of the size of the particles of digesta. When Ru-P in conjunction with Cr-EDTA, was continuously infused intraruminally for 9 d,

ruthenium was detected in faecal samples from day 1 to day 16 with the excretion plateau between days 4 and 11.

The effect of Ru-P on the rumen's microbial population was assessed by measuring production rates of volatile fatty acids *in vitro* in samples of whole rumen digesta. At Ru-P concentrations of 10^{-5}M and 10^{-4}M the production of acetic, propionic and butyric acids was the same as that observed in an untreated control. At 10^{-3}M however, acetic and propionic acid production was severely inhibited whereas that of butyric acid was unaltered. Effects on microbial metabolism were expected because of the toxic nature of most metals to bacteria (Kotb and Luckey, 1972).

The conclusions of Evans *et al* (1977), which have been supported by others (Macrae, 1974; Faichney, 1975b; Beever *et al*, 1978), identified ^{103}Ru-P as the most appropriate solid phase component of a dual-phase marker system in situations where radioactive isotopes could be easily used. The increased marker volume and concentration, required by the less sensitive X-ray spectrophotometric analytical procedure, meant that unlabelled Ru-P had specific disadvantages. Nevertheless, Ru-P in conjunction with a suitable non-radioactive marker of the liquid phase of digesta provided the best means by which flow rate could be estimated in animals provided with simple cannulas and housed in facilities where the use of radioisotopes was prohibited.

Ru-P, in radioactive or inert form, has been used exclusively as the solid phase marker component of a two marker system with Cr-EDTA. Applications of this widely used marker system are reviewed in Section 2.2.3.2.3 below. However, in a study of the measurement of the rate of flow of digesta in sheep fed either continuously

or once daily, Faichney (1980), while using ^{103}Ru-P and ^{51}Cr-labelled Cr-EDTA (^{51}Cr-EDTA) together, calculated the rates of flow which would have been obtained had either marker been used in isolation. Compared with the rates of flow calculated using both markers, ^{103}Ru-P underestimated digesta flow by up to 30.5% and N flow by up to 23.1%. This error arose because the Ru-P was associated with the particulate fraction of digesta, and the samples obtained from the abomasal cannulas of the sheep contained particular matter in greater proportion than the intestinal digesta from which they were taken.

Other particulate phase markers

Internal markers, indigestible components of the diet which can be detected in the digesta passing through the intestinal tract, have attracted attention as solid phase components of two-marker systems (Kotb and Luckey, 1972). Lignin is the component most frequently used, and Hogan and Weston (1967) in one of the earliest attempts to counter non-representative sampling problems, used this component and ^{51}Cr-EDTA together to measure rates of flow of chopped and ground roughages fed to sheep. They considered that the lignin marked the larger particles of digesta (those contained by a coarse terylene filter) whereas the ^{51}Cr-EDTA was closely associated with soluble substances and fine, suspended, particles.

Crickenberger et al (1979) used lignin alone as an indigestible marker of the passage of digesta from the abomasum of steers fed corn silage, making no provision for differential solid and liquid flow rates or for biased sampling. The use of lignin as an indigestible marker must be questioned because of the indeterminate

digestion of this fraction which may occur during passage through the ruminant gastrointestinal tract. Miswicki, Owens, Poling and Burnett (1980) observed digestibility coefficients of between 0.16 and 0.30 for lignin when feeding a poor quality hay to ruminally cannulated steers.

More recent refinements of the indigestible internal marker technique have been more precise in their definition of the marker substance. Thus Waller et al (1980) used indigestible neutral detergent fibre (INDF) and indigestible acid detergent fibre (IADF) as alternative solid phase markers in abomasal flow rate studies. Neutral detergent and acid detergent fibre levels were measured in the particulate fraction, separated by centrifugation, of samples of abomasal digesta. IADF and INDF levels were measured in the diet by subjecting food samples to acid-pepsin digestion in vitro, to simulate digestion in the abomasum, before extracting the residual fibre component.

2.2.3.2.2 Liquid phase markers

Polyethylene glycol

In addition to its use as an indicator of digestibility (Kotb and Luckey, 1972), and as a corrective marker in measuring flow rates by total collection (Corse and Sutton, 1971), polyethylene glycol (PEG) has been used as a marker of the liquid or water phase of digesta in experiments in which digesta flow was measured indirectly (Goshtasbpour-Parsi et al, 1974; Coleman, Dawson and Grime, 1980; Waller et al, 1980). The polymerised diol is produced through the reaction of ethylene oxide with water, giving functional hydroxyl groups which allow the continuation of the reaction (Kotb and Luckey, 1972). The PEG produced is water-soluble, its physical

form being determined by the degree of polymerisation and hence the average molecular weight of the PEG molecules in any particular mixture. PEG compounds with molecular weight up to 600 are liquids, whereas those with molecular weights greater than 1000 are solid (Kotb and Luckey, 1972).

PEG has now been generally discredited as an indirect flow-rate marker. There have been many reports of incomplete recovery of the material in faeces, indicating some degree of intestinal absorption or metabolism (Downes and McDonald, 1964; see Kotb and Luckey, 1972). The fact that flow rates determined by correcting 'total' digesta collections for incomplete marker recovery are higher when PEG is used instead of Cr_2O_3, is also indicative of absorption of the former marker before the point of sampling (Corse and Sutton, 1971). Poor and inconsistent recovery has also been attributed to the lack of a sensitive, specific method for determining PEG. The turbidimetric procedure of Ulyatt (1964) is the technique most often employed (Kotb and Luckey, 1972; Goshtasbpour-Parsi et al, 1974; Waller et al, 1980). Because of these problems, the quantity of PEG which must be used is relatively large, and radiolabelled ^{3}H-PEG and ^{14}C-PEG have both been used to allow a rapid, sensitive means of analysis (Macrae, 1974).

Another concern about the use of PEG as a liquid phase marker was demonstrated at the Hannah Research Institute, Ayr, by Czerkawski and Breckenridge (1969). Following in vivo experiments in which the use of PEG had yielded unreasonable estimates rumen volume and digesta flow rates, these workers investigated the distribution of PEG in aqueous buffer solutions containing suspended food particles. Dilution of the marker within the total water volume was not uniform,

the marker being excluded from the water contained within the swollen food particles. An equation could be produced which, for a given food, allowed the theoretical marker concentration to be calculated from the observed concentration, the weight of food in the suspension and the total volume of water present. Such parameters could obviously not be measured in vivo. The authors demonstrated that an observed concentration twice that of the theoretical concentration could easily be obtained, leading to gross underestimation of rumen volume or digesta flow rate.

When using PEG and ^{51}Cr-EDTA simultaneously to measure rumen water volume, Downes and McDonald (1964) obtained identical estimates with each marker. However, less than 85% of the PEG which was administered could be recovered in the faeces and none could be detected in the urine.

Chromium complex of ethylenediamine tetraacetic acid

The principal alternative to PEG as a water-soluble marker in nutritional studies has been Cr-EDTA (Kotb and Luckey, 1972). This marker was suggested and developed at the same CSIRO unit in Australia where the particulate marker, Ru-P, originated (Downes and McDonald, 1964). In their initial work, Downes and McDonald (1964) evaluated the radio-labelled ^{51}Cr-EDTA complex as a marker of intestinal fluid to estimate rumen volume. Their experiments involved the administration of small amounts (0.1 g, 30 - 60 μCi) of ^{51}Cr-EDTA and PEG (6 - 10 g) together in approximately 400 ml of aqueous solution into the rumen. Up to 12 h after the markers had been given, the observed ^{51}Cr:PEG ratios in rumen liquor samples were not significantly different from those which would have been predicted from the known infusions. Estimates of rumen volume

were therefore the same irrespective of the marker used in the calculation. Between 85 and 95% of ^{51}Cr-EDTA administered as a single dose orally or intraruminally was recovered in the faeces in the first 9 - 10 days. A small proportion (4.7% maximum) of the marker always appeared in the urine. The excretion of ^{51}Cr-EDTA administered intravenously was both rapid (most occurring in the first day after the marker was injected) and complete (90% of the ^{51}Cr-EDTA appeared in the urine with none being detected in the rumen or faeces). The Australian workers concluded that the relatively low hazard presented by ^{51}Cr, the ease with which the material's gamma emissions could be detected and the qualities which they had demonstrated, rendered the complex a suitable marker for use in ruminant nutrition.

Subsequent work (Binnerts, Van't Klooster and Frens, 1968) has suggested that the unlabelled complex (Cr-EDTA) may be used in the same manner as the radio-labelled one if suitable analytical procedures are available for the detection of chromium. They described an atomic absorption spectrophotometric technique for this purpose and observed faecal loss, and concomitant urinary excretion, of less than 5% when the marker was administered to sheep and cattle. The non-labelled Cr-EDTA was prepared by reacting chromic chloride ($CrCl_3.6H_2O$) with an excess of the sodium salt of ethylenediamine tetraacetic acid (Na_2EDTA) and adjusting the pH to 7 with sodium hydroxide. The method of Downes and McDonald (1964) reacts excess ^{51}Cr-labelled $CrCl_3.6H_2O$ with Na_2EDTA and adjusts the pH with ammonium hydroxide. When this procedure has been scaled up to produce the unlabelled marker, the considerable quantity of ammonium chloride in the infused solution has markedly

altered ruminal ammonia levels (Macrae, 1974). One hour after administering 100 ml of Cr-EDTA solution prepared in this manner, Macrae (1974) recorded an increase in ammonia concentration in the rumen liquor from 25 to 225 mg NH_3 l^{-1}. The importance of the conditions of the complexing reaction are now appreciated and recent work has used the safe procedure of Binnerts et al (1968).

In addition to slight absorption from the ruminant gastro-intestinal tract, non-ideal behaviour of Cr-EDTA has also been shown by Warner (1969) who demonstrated that under certain conditions, ^{51}Cr-EDTA may bind with particulate matter in the rumen.

In ruminant nutrition, Cr-EDTA and ^{51}Cr-EDTA have been used, almost exclusively, as liquid phase markers to complement Ru-P or ^{103}Ru-P respectively in two-marker or dual-phase marker systems. This use is dealt with in Section 2.2.3.2.3 below. However, in the period between the development of ^{51}Cr-EDTA and ^{103}Ru-P as viable markers, Hogan and Weston (1967) used ^{51}Cr-EDTA to mark the liquid phase of ovine digesta while employing lignin as an internal particulate phase marker.

2.2.3.2.3 Simultaneous use of ^{103}Ru-P with ^{51}Cr-EDTA and Ru-P with Cr-EDTA

Apart from the exceptions mentioned above, Ru-P (or ^{103}Ru-P) and CR-EDTA (or ^{51}Cr-EDTA) have usually been used together as the components of two-marker or dual-phase marker systems for indirect measurement of the flow rate of digesta in cannulated ruminants (Beever et al, 1978). Their use was encouraged by a desire among many research workers to use simple, less debilitating, surgical preparations (Faichney, 1975b; Macrae, 1975; Macrae and Wilson, 1977; Beever et al, 1978) coupled with a recognition that

unrepresentative sampling from simple cannulas required the use of two markers.

Following the development of ^{103}Ru-P, that marker and ^{51}Cr-EDTA have been used extensively by the CSIRO in Australia (Faichney, 1975b). In these studies a solution containing 1 μCi ^{51}Cr-EDTA and 0.2 μCi ^{103}Ru-P ml^{-1} was routinely infused into the rumen of sheep at a rate of about 48 ml d^{-1}. The infusion was preceded by a priming dose, designed to reduce the time required for marker concentrations to reach equilibrium levels, and marker concentrations usually stabilised about four days after continuous infusion started. This group of workers have used the markers to measure the rates of flow of digesta in sheep fed either continuously or frequently at fixed intervals. When digesta were taken from the abomasum, one quarter of all samples were grossly unrepresentative of the material in the tract (^{103}Ru : ^{51}Cr <0.9 or >1.1). One third of all ileal samples were similarly biased. For both abomasal and ileal samples, the mean ^{103}Ru : ^{51}Cr concentration ratio was greater than unity, indicating a general tendency to acquire particulate matter in amounts greater than were required. In these studies, the Australian workers used the observed marker concentrations to determine the true composition of the digesta. This double-, or dual-phase, marker technique does not require that each marker should associate exclusively with one phase of digesta.

When investigating the effect of formaldehyde treatment on the passage of solutes and particulate matter in the gastrointestinal tract of sheep, Faichney (1975a) infused the ^{103}Ru-P/^{51}Cr-EDTA mixture at each of three points. Overall, when

administered intra-ruminally, ^{103}Ru-P took much longer than ^{51}Cr-EDTA to traverse the whole tract. From the abomasum, the same relationship remained and the small difference in transit times (35 h and 32 h for ^{103}Ru-P and ^{51}Cr-EDTA respectively) was statistically significant. Mean retention times for the two markers in the hind gut were, however, similar, indicating that the liquid and particulate phases of digesta did not behave independently in that part of the gut.

Following the development of the unlabelled markers and of suitable analytical procedures to determine ruthenium and chromium in biological materials (Macrae and Evans, 1974; Evans et al, 1977), Ru-P with Cr-EDTA has been used as a marker system at the Hill Farming Research Organisation (Evans et al, 1977) and at the Grassland Research Institute (now the Animal and Grassland Research Institute), Hurley (Beever et al, 1978; Beever, Ulyatt, Thomson, Cammell, Austin and Spooner, 1980; Ulyatt, Beever, Thomson, Evans and Haines, 1980).

The work of Beever et al (1978) has already been mentioned in Section 2.2.3.2.1. They examined the relationship between ruthenium and chromium concentrations, expressed as proportions of the daily infusates, in 48 digesta samples taken from calves fitted with simple cannulas. They found that the two concentrations were directly related but that at high chromium levels the ruthenium concentration was disproportionately high whereas, at low chromium levels, less ruthenium was present than would have been expected in an unbiased sample.

These workers were also concerned to establish that the Ru-P and Cr-EDTA, administered in much greater amounts than had been

used previously with the radio-labelled markers, would not contravene criterion (vi) of Kotb and Luckey (1972; see Section 2.2.3.2 above) and affect the rumen microbial population. Possible effects were assessed by determining, in vitro, the digestion of the organic matter of a barley-based diet. Measurements were made with Ru-P and Cr-EDTA added to the buffered rumen liquor incubation mixture, either alone or together, to give ruthenium concentrations of 0, 4.0×10^{-6}, 2.2×10^{-5}, and 4.0×10^{-5}M and chromium concentrations of 8.0×10^{-5}, 4.4×10^{-4} and 8.0×10^{-4}M. At the concentrations tested, chromium had no measurable effect on the digestion of organic matter whereas ruthenium, at concentrations $>2.2 \times 10^{-5}$M depressed organic matter digestiblity by about 15%. In subsequent experiments when the markers were infused intraruminally, Beever et al (1978) calculated that the concentrations of ruthenium and chromium in the rumen liquor, resulting from the infusion of 12 mg Ru and 280 mg Cr d^{-1} DM, were similar to the lowest levels tested in vitro. It is not clear whether the volume infused was 240 or 480 ml d^{-1}. These workers used the methods described by Faichney (1975b) to estimate the true composition of digesta and hence to determine the flow rates of nutrients. However, they appear to have separated the sampled digesta into two components, a residue and a supernatant, by centrifugation, and then to have determined only the ruthenium content of the particulate residue and the chromium concentration in the liquid fraction. The double-marker technique requires that the concentrations of both markers be determined in two samples whose solid: liquid ratios differ (Faichney, 1975b).

The same markers were used at the Grassland Research Institute in a series of studies on the nutrient supply from fresh forages fed to growing calves. In the first (Beever et al, 1980), Ru-P with Cr-EDTA was used to measure the rate of flow of digesta at the duodenum of calves fed fresh, harvested, ryegrass or white clover. The second study (Ulyatt et al, 1980) attempted to measure similar parameters at pasture. Ru-P and Cr-EDTA were again chosen as the markers, and a miniaturised portable peristaltic pump together with a portable apparatus for sampling digesta were developed to allow, respectively, continuous infusion of the markers and frequent automatic sampling of duodenal digesta from the T-piece cannula. Flow rates of organic matter and non-ammonia N were measured, but no direct estimates of herbage intake were obtained. However, assuming similar compartmentalisation of digestion as had been observed in the previous study indoors (Beever et al, 1980), Ulyatt et al (1980) were able to calculate theoretical nutrient flows per unit digestible organic matter intake. These values agreed closely with those for calves eating harvested forage indoors.

At the Hill Farming Research Organisation, ^{103}Ru-P with ^{51}Cr-EDTA and Ru-P with Cr-EDTA are both used in digestion and metabolism studies with fistulated ruminants (Evans et al, 1977; Macrae, Milne, Wilson and Spence, 1979). When considerations of safety regulations and restrictions are not paramount, the radio-labelled markers, with their property of being rapidly determined, are preferred. In work conducted in areas without appropriate clearance, Ru-P and Cr-EDTA are employed.

Faichney and Griffiths (1978) studied the behaviour of ^{103}Ru-P and ^{51}Cr-EDTA in the stomach (reticulo-rumen, abomasum and omasum) of sheep fed continuously. Although ^{103}Ru-P was strongly adsorbed onto particulate matter and did not appear in solution, exchange of this marker occurred between large and small particles. The authors therefore concluded that the mean retention time for particulate matter in the rumen could not be simply obtained using Ru-P as an adsorbable marker. In these studies of retention times the ^{103}Ru-P and ^{51}Cr-EDTA were administered in a single dose (see Faichney, 1975b) at 09.00 h, with frequent sampling for periods up to 96 h after marker administration.

2.2.3.3 Sites of cannulation for indirect measurement of the flow rate of digesta

When assessing the flow of digesta, and the supply of nutrients, to the absorptive regions of the small intestine of the ruminant animal, two sites, the abomasum and the proximal duodenum, are suitable for the siting of T-piece cannulas (Faichney, 1975b; Thomas, 1978). Some workers consider that output from the forestomach (i e the reticulo-rumen) is the parameter of most interest (Sutton and Oldham, 1977). For this, cannulation of the omasum would be most appropriate but, although techniques exist for this (Willes and Mendel, 1964), the operation is difficult (Thomas, 1978) and most workers again rely on cannulas in the abomasum or proximal duodenum (Sutton and Oldham, 1977; Thomas, 1978).

It is difficult to ascertain which of the two sites is the more popular for T-piece cannulation. The majority of references encountered during the preparation of this review described the use of duodenal preparations. However, this total included work with animals fitted with re-entrant cannulas and this preparation

cannot be positioned at the abomasum (Thomas, 1978). The abomasum and duodenum each have particular attractions as sites for the insertion of T-piece cannulas.

Cannulation at the abomasum avoids disruption of the duodenum's role in neural and endocrine regulation of the functioning of the gastrointestinal tract (Faichney, 1975b). The contribution of endogenous secretions to the sampled digesta is less if the cannula is placed in the abomasum rather than the duodenum, particularly when the latter preparation is anterior to the common bile and pancreatic ducts (Harrop, 1975; Thomas, 1978). Continuity of flow, or at least the ability to acquire a sample at a given time, should also be superior with abomasal preparations because of the less episodic nature of digesta movement through this organ (Wenham and Wyburn, 1980).

The major advantage claimed for duodenal cannulation is that it allows the acquisition of a representative sample of digesta and that consequently only one indigestible marker need be used (Thomas, 1978). This has also been reported for a T-piece cannula inserted at the terminal ileum (Hogan, 1973). However, Macrae (1974) and Beever et al (1978) have demonstrated non-representative sampling from T-piece duodenal cannulas. In both cases this was done by comparing the concentrations of the two markers (^{103}Ru-P/^{51}Cr-EDTA for Macrae (1974); Ru-P/Cr-EDTA for Beever et al (1978)) in the digesta samples. Macrae (1974) observed higher than expected ruthenium concentrations, indicating a sampling bias in favour of particulate material. Beever et al (1978) encountered the same bias at low flow rates.

2.2.3.4 Schedules for sampling digesta

When taking 'spot' samples of digesta from a T-piece cannula, two methods may be used, either independently or together, to ensure that the final bulked sample is representative of the digesta which have passed the cannula in a 24 h period. These techniques are continuous feeding, designed to abolish any diurnal pattern in the flow of digesta (Sutton and Oldham, 1977; Faichney and Griffiths, 1978; Thomas, 1978; Waller et al, 1980), and very frequent sampling, allowing the 24 h period to be considered as the steady state period (Corse and Sutton, 1971; Goshtasbpour-Parsi et al, 1974; Faichney, 1980; Morgan et al, 1980; Ulyatt et al, 1980).

Continuous feeding has been adopted in several studies, with the aim of reducing the frequency with which samples must be collected (Sutton and Oldham, 1977). These workers suggested that frequent feeding would allow three samples per day over three days to be adequately representative. Waller et al (1980) fed a corn-based diet continuously to abomasally cannulated steers at a rate of 303 g DM h^{-1}. Digesta samples were collected at 24, 48 and 72 h intervals. Although the standard errors of mean nutrient flows calculated from the 24 h-frequency samples were the lowest, the sampling interval or frequency did not significantly affect the calculated rate of flow. Since the sampling time was the same (11.00 h) regardless of sampling interval, no account could be taken of diurnal variation. In this context, it is interesting to note that Faichney (1978) detected distinct diurnal fluctuations in net water movement from the rumen of sheep which were fed continuously under conditions of continuous light. The

animal house did have windows and the automatic feeders were loaded at the same time each morning thus providing the sheep with evidence of a 24 h cycle. Another possible caution against the use of continuous feeding is the effect that continuous provision of the diet may have on the digestion and metabolism of nutrients (Thomas, 1978). If such effects are expected, experimental feeding should be less frequent to provide results of relevance to commercial practice (Thomas, 1978).

In experiments in which feeding has been less frequent, many more samples have been collected. Thus Corse and Sutton (1971), when feeding hay and concentrate diets to sheep twice daily, constructed six samples to represent every two hours of the 12 h steady-state feeding cycle. The six samples were collected over a period of three days to avoid disruption of normal physiology by the rapid removal of a large amount of digesta. Goshtasbpour-Parsi et al (1974) sampled even more frequently - 18 times in 24 h - when feeding pelleted rations to omasally and abomasally cannulated lambs. The feeding interval was 12 h but the 24 h period was considered to be the steady-state unit. Sampling intervals were not constant, with frequency being increased before and after each feeding. This rigorous sampling regime was maintained for 10 d. Morgan et al (1980) sampled at two hour intervals for a period of 24 h when feeding supplemented silage diets at 09.00 h and 17.00 h to abomasally cannulated wethers. A further refinement of this procedure was described by Ulyatt et al (1980) who used an automated digesta collection apparatus when measuring the flow of nutrients to the duodenum of grazing calves. They were able to vary the interval between samples from 100 to 900 sec and the

sampling duration from 1 to 6 sec. Two 24 h sampling periods were used for each animal.

2.2.4 Verification of the normality of cannulated animals

The experiments thus far described have all been conducted with animals subjected to surgical modification of the gastro-intestinal tract. If the results of such work are to be of more than academic interest, the functional normality of the experimental animals must be confirmed (Sutton and Oldham, 1977; Egan and Ivan, 1979; Harrison, 1979). A dilemma at once presents itself: surgical modifications are performed to allow measurement of digestive processes in ruminant animals, and yet the absolute accuracy of the values obtained cannot be assessed since the modifications are a necessary pre-requisite to the acquisition of the values.

Measures of overall normality can be performed by monitoring whole-body functions which can also be determined in intact animals. The digestibility of the diet has therefore been measured on several occasions (Putnam and Davis, 1965; Macrae and Wilson, 1977), the general finding being that neither rumen, simple small intestine nor re-entrant small intestine cannulation causes measurable alteration in the parameter.

One of the more detailed studies of this type was performed at the Hill Farming Research Organisation by Macrae and Wilson (1977). Over a period of 12 months they monitored voluntary intake of food, digestibility of dry matter, nitrogen balance, the concentrations of corticosteroid, aspartate, aminotransferase, protein bound iodine, glucose and urea in venous blood, rates of wool growth and mean marker retention times in the rumen and caecum, in a group of 24 Scottish Blackface wethers. After an initial

measurement phase, eight sheep were left intact and 16 were fitted with permanent rumen cannulas. The digestion and metabolism determinations were repeated, before eight of the rumen cannulates were fitted with simple 'T-piece' duodenal and ileal cannulas and the other eight with re-entrant cannulas at the same positions. Once intake, digestion and metabolism determinations had again been performed, the retention times of flow rate markers within the rumen were determined for intact and fistulated sheep. The workers were unable to demonstrate significant differences in dry matter digestibility, N balance, blood parameters or mean marker retention times, attributable to surgical intervention of varying severity. Rates of wool growth were monitored as indices of the utilisation of metabolisable nutrients and, in this case, Macrae and Wilson (1977) were able to detect differences due to surgery. The fitting of rumen, and simple duodenal and ileal, cannulas had no effect whereas sheep fitted with re-entrant cannulas had their wool growth reduced by almost 50%. This observation is in agreement with the findings of experiments conducted in non-ruminant animals (Richards, Drury, Goll, Bessent and Al-Shamma, 1978). Using indirect calorimetric techniques, these workers demonstrated increases in energy expenditure ranging from 10% in response to minor elective surgery, to 125% after extensive full skin thickness burns. The hypermetabolism appeared to be the result of increased catabolism of protein to provide gluconeogenic intermediaries for the Kreb's cycle but could not be reversed by intravenous infusion of glucose.

With surgical preparations designed to allow measurement of digesta flow, it is obviously of prime importance that flow

is not disrupted (Macrae and Wilson, 1977; Sutton and Oldham, 1977). Unaltered rates of flow or retention times of markers can provide some reassurance that this is the case. More recently, workers at the Rowett Research Institute in Aberdeen investigated the changes in gastrointestinal tract function following upon the establishment of a range of intestinal cannulas (Wenham and Wyburn, 1980). They employed sophisticated radiological procedures, using barium sulphate as a radio opaque dye, to observe and record gut motility patterns in intact sheep and in animals fitted with 'T-piece', 'Ash-type' re-entrant or non-transecting re-entrant cannulas in the ascending duodenum, transverse duodenum, transverse jejunum or teminal ileum. The authors first confirmed that the normal pattern of gut motility, in the region from the abomasum to the ileo-caecal junction, was unaffected by the insertion of a small (7 mm external diameter) catheter into the abomasum to facilitate administration of the barium sulphate. All other preparations caused measurable alteration in the manner in which digesta was moved through the small intestine. The simplest preparation, with a 'T-piece' cannula fitted in the transverse duodenum, resulted in the least dysfunction. Even in this case, however, there was a tendency for a pool of digesta, resistant to clearance by the advancing wave of peristaltic contraction, to remain in the region of the cannula and leak back into the duodenal bulb. The 'Ash-type' re-entrant cannula in the ascending duodenum was also relatively free from major influences on digesta flow. However, not all duodenal contractions were sufficiently powerful to push enough digesta through the cannula to propagate a peristaltic surge. Four months after surgery, the duodenal bulb had increased to

one and a half times its original size. All other duodenal re-entrant preparations afforded appreciable resistance to the passage of digesta with the consequence that the region of the tract anterior to the cannula became distended, backflow of digesta to the duodenal bulb occurred and leakage from the cannula was a problem. Re-entrant cannulation of the jejunum resulted in similar problems and transfer of a bolus of digesta from the duodenum to the jejunum, which took only 'seconds' in intact animals, could take up to 30 min. The region of the tract anterior to the cannula was always full of digesta, contrasting with the compartmentalised distribution in untreated animals. The fitting of re-entrant ileal cannulas also introduced severe problems, with the site of the cannula offering great resistance to the passage of digesta and to the propagation of the advancing wave of contraction, which could not be detected posterior to the cannula. Simple cannulation at this site did not impede the flow of digesta to the same extent, but the preparation did deteriorate with age.

It is obvious from work such as this, which appears to be the most thorough investigation yet conducted of the normality of surgically prepared animals, that great care must be exercised when choosing a preparation for investigating the functioning of the digestive tract. The fact that similar values, for given parameters, are obtained with surgically prepared and intact animals, does not, by extrapolation, mean that measurements made using the cannula are similarly unaffected by the surgery required for its insertion.

2.3 DEGRADABILITY OF DIETARY NITROGEN

2.3.1 Introduction

In ruminant animals dietary protein may be extensively degraded to peptides, amino acids, ammonia and the carbon skeletons of amino acids by the action of the symbiotic microbes in the reticulorumen (Smith, 1975; Armstrong, 1980). The extent of this degradation, and the concomitant synthesis of bacterial and protozoal protein, determines the nature and amount of the protein available for subsequent digestion and absorption by the host animal (Smith, 1975; Armstrong, 1980).

The fact that such microbial intervention invalidates the use of an overall coefficient of digestibility to assess the nutritional value of dietary protein (i e a digestible crude protein system (Evans, 1960)) has been recognised (Armstrong, 1973; Buttery, 1977; Armstrong, 1980). This, in turn, has led to the development of a series of 'models' which attempt to describe the extent of degradation of dietary protein or nitrogen in the rumen and the supply of microbial and residual dietary N to the small intestine of ruminant animals (Roy , Balch, Miller, Ørskov and Smith, 1977; Agricultural Research Council, 1980). Consideration may also be given to the contribution of endogenous N to the profile of nitrogenous compounds presented at the small intestine (ARC, 1980).

The degradability of dietary N may be defined as:

$$1 - \frac{\text{(duodenal N - duodenal microbial N - duodenal endogenous N)}}{\text{dietary N}}$$

(ARC, 1980). Measurement of the intake of dietary N presents few problems and, provided that the flow rate of the digesta can be accurately determined (see Section 2.2), measurement of duodenal N

is also straightforward (ARC, 1980). The measurement of microbial and endogenous N is more complex, and techniques which have been investigated are described in Sections 2.3.2 and 2.3.3 below. Since measurement of true degradability presents considerable technical difficulty, attempts have been made to find simpler indices of its magnitude (ARC, 1980; see Section 2.3.4).

2.3.2 Microbial markers

2.3.2.1 Endogenous microbial markers

Several techniques, designed to quantify the proportion of microbial nitrogen in the digesta reaching the absorptive regions of the ruminant gastrointestinal tract, have used endogenous markers (Hutton, Bailey and Annison, 1971; McAllan and Smith, 1972; Czerkawski, 1974; Ling and Buttery, 1976, 1978). Such compounds may be components of bacteria or protozoa, but which are absent from the food and any intestinal secretions (Ibrahim, Ingalls and Bragg, 1970; Hutton et al, 1971). Alternatively, they may be substances which are present in the gut microbiota and in the food, provided that all of the compound detected in the digesta sample can be considered to be of microbial origin due to intra-ruminal degradation of the dietary component (McAllan and smith, 1972). The chosen marker must be readily detectable in the digesta sample and a constant, or measurable, relationship should exist between the amount of marker and the total amount of microbial N.

2.3.2.1.1 2,6 Diaminopimelic acid

The amino acid 2,6 diaminopimelic acid (DAPA) was discovered in 1951 (El-Shazly and Hungate, 1966). It has since been isolated from most species of bacteria and blue-green algae (El-Shazly and Hungate, 1966) and has been identified as a constituent amino

acid of the mucopeptides of the cell wall of many bacterial species found in the gastroinestinal tract of ruminants (Mason and White, 1970). This amino acid is generally considered to be absent from higher plant and animal tissues (Czerkawski, 1974). Harrison, Beever, Thomson and Osbourn (1973) and Ling and Buttery (1978) reported that they were unable to detect DAPA in the diets which they fed to sheep. DAPA has therefore been proposed as a suitable marker for bacterial nitrogen in digesta (El-Shazly and Hungate, 1966; Mason and White, 1970; Czerkawski, 1974).

The ratio of DAPA (or DAPA N) to total N is not constant for all bacterial species and it may occasionally be completely absent, since its presence in the mucopeptide of the cell wall is mutually exclusive with that of lysine (Czerkawski, 1974). However, experimenters employing this technique have generally contended that with constant feeding and environment, a stable bacterial population, with a characteristic DAPA: total N ratio, will be established (El-Shazly and Hungate, 1966; Czerkawski, 1974). Hutton et al (1971) fed a constant diet to a single fistulated wether for a period of three months during which time they collected 10 samples of rumen bacteria. The mean DAPA:total N ratio was 0.052 ± 0.0033, and the workers considered that this was sufficiently precise to allow the ratio to be regarded as constant given regular feeding intervals and a common sampling time. Czerkawski (1976) reported that the DAPA content of bacterial isolates from sheep eating differing proportions of hay and concentrates was independent of diet. However, he also observed that the concentration of DAPA was greater in large than in small bacteria. The two bacterial fractions were separated from strained rumen liquor by differential centrifugation. Ling and Buttery

(1978) used DAPA as a bacterial marker in an experiment using three fistulated sheep and three isoenergetic, isonitrogenous diets in a Latin square design. Nine individual DAPA nitrogen:total nitrogen ratios were thus obtained and these yielded a mean value of 6.75 $\pm$ 1.768. These marked variations (coefficient of variation = 26.73%) could not be explained by animal, diet or period effects. Ling and Buttery therefore used an individual marker:total N ratio for each animal x diet combination. Mercer, Allan and Miller (1980) encountered similar DAPA N:microbial N variation in an experiment in which the extent of ruminal degradation of the nitrogenous component of a range of diets was assessed. They adopted the same solution, using multiple DAPA N:microbial N ratios.

The DAPA content of digesta or microbial samples can be determined by several methods. El-Shazly and Hungate (1966) described a technique in which centrifuged rumen contents were hydrolysed in boiling hydrochloric acid and the filtered hydrolysate was passed through a large chromatographic column. The eluted DAPA reacted with ninhydrin, and no other ninhydrin-reactive amino acids appeared at the same time to interfere with the colorimetric determination. Similar procedures have been used by Smith, McAllan, Hewitt and Lewis (1978). Czerkawski (1974) proposed a simpler technique which allowed more rapid purification and subsequent chromatography of the hydrolysed sample. Again, use has been made of similar techniques (Hutton et al, 1971; Mercer et al, 1980). DAPA can also be determined along with other amino acids, using an autoanalyser (Czerkawski, 1976; Harrison et al, 1973).

Although some workers have reported the absence of DAPA in the foodstuffs they have used in ruminant studies, others have

detected it. Czerkawski (1974) detected 0.15, 0.05 and 0.11 g DAPA kg^{-1} DM in ground samples of hay, dried grass and barley respectively. These values compared with 2.15 - 3.60 g DAPA kg^{-1} DM for rumen bacteria. DAPA has also been detected in rumen protozoa (Czerkawski, 1976; Ling and Buttery, 1978), although the levels may be so low as to be of no practical significance (Ling and Buttery, 1978).

2.3.2.1.2 2-Aminoethylphosphonic acid

2-Aminoethylphosphonic acid (AEPA) was first isolated from rumen protozoa by Horiguchi and Kandatsu (1959). This was the first compound, containing a direct C - P bond, to be extracted from animal material . This bond is characteristically resistant to hydrolysis by mineral acids, and alkylphosphonates, as these compounds are generally known, can usually be determined as non-hydrolysable phosphorous (Miceli, Henderson and Myers, 1980). However, the C - P bond can be hydrolysed by perchloric acid and, in this event, AEPA can be measured as phosphate following hydrolysis of a sample which has previously been hydrolysed in hydrochloric acid (Czerkawski, 1974). Horiguchi and Kandatsu (1959) were unable to state whether AEPA was widely distributed in nature or occurred only in rumen protozoa. It is now known that although higher vertebrates, in particular, have no significant metabolic involvement with alkyphosphonates, AEPA and related compounds are found in many invertebrate, and some vertebrate, species as components of lipids and proteoglycans (Czerkawski, 1974; Ling and Buttery, 1978; Miceli et al, 1980). Contamination of animal and plant material with AEPA of protozoal origin may also occur (Czerkawski, 1974; Ling and Buttery, 1978).

The success with which AEPA has been isolated from ruminant digesta and microbial samples has been variable (Ibrahim et al, 1970; Czerkawski, 1974; Ling and Buttery, 1978). Ibrahim et al (1970) attempted to measure the distribution of a range of amino acids in food fed to, and rumen contents, rumen bacteria and rumen protozoa acquired from, four fistulated dairy cows. All samples were hydrolysed in hydrochloric acid and subsequently resolved on a Beckman model 116 amino acid analyser. The authors identified a peak, eluted after 185 min, which corresponded with the elution of a standard solution of AEPA. However, a subsequent publication (Anon, 1973) stated that 1,2 diaminoethylphosphonic acid had been used, in error, as the standard in the original paper. AEPA had an elution time of 27 - 28 min and, under the conditions used, could not be separated from other amino acids. Ling and Buttery (1978) hydrolysed their digesta and microbial samples in a similar fashion and separated the AEPA from other amino acids using an Evans model 1294 automatic amino acid analyser. Using this system, AEPA was eluted as a single symmetrical peak with an elution time of 52 min.

Although several workers have analysed biological material for AEPA, and have suggested its use as an endogenous marker of ruminal protozoa, the comparative study of Ling and Buttery (1978) is one of the few in which this compound has been tested as a microbial marker. As with the DAPA used in the same study (see Section 2.3.2.1.1 above), considerable variation in the ratio of marker N:total N (in this case AEPA N:protozoal N) was observed. This might not preclude the use of the acid as a protozoal marker if individual determinations of the ratio could be made for each

animal x diet combination. However, Ling and Buttery (1978) also found appreciable amounts of AEPA in each of the three diets (barley grain - and barley straw-based diets with white fish meal, soya-bean meal or urea as the main source of nitrogen) and in rumen bacteria. These observations, and the fact that calculations of the contribution of protozoal N to duodenal N flow therefore gave unrealistically high values, led the authors to discount AEPA as a valid marker of protozoal nitrogen.

2.3.2.1.3 Nucleic acids

The nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are constituents of all prokaryotic and eukaryotic cells (Yudkin and Offord, 1975). They play a central role in the storage of genetic information, its transference from generation to generation (DNA) and in the translation of encoded genetic information into ordered growth of the cell (DNA and RNA) (Whitehouse, 1975; Yudkin and Offord, 1975).

Both groups of compounds are polymers, the monomer unit of which may be considered to be a nucleotide (Bell, Davidson and Scarborough, 1968). Each nucelotide consists of a purine or pyrimidine base linked to a pentose sugar which is in turn esterified to phosphoric acid (Bell et al, 1968). In RNA, the pentose sugar is ribose, and the bases are cytosine (2 hydroxy 4-amino pyridine), uracil (2,4 dihydroxy pyridine), adenine (6 aminopurine) and guanine (2 amino-6 hydroxy purine). In DNA, the sugar is deoxyribose, and thymine (5 methyl uracil) occurs in place of uracil (Bell et al, 1968).

DNA is concentrated in the nucleus (in eukaryotes) or nuclear zone (in prokaryotes) of the cell, where up to 99% of total cell

DNA is associated with approximately equal amounts of protein whereas in bacteria only small amounts of protein are present with the nucleic acid (Whitehouse, 1975). Some RNA is also found in the nucleus/nuclear zone (messenger RNA (mRNA), the intermediate carrier of genetic information from the DNA pool in the nucleus to the sites of protein synthesis), but the bulk of RNA exists in the cytoplasm. The largest single fraction is ribosomal RNA (rRNA), which constitutes the bulk of bacterial RNA. This rRNA is associated with protein and is the site of synthesis of cell protein. The other extra-nuclear RNA fraction is amino-acyl transfer RNA (tRNA), responsible for transferring amino acids to the mRNA/rRNA complex for protein synthesis (Yudkin and Offord, 1975). The levels of rRNA are generally stable and the polymer is not rapidly synthesised or degraded. In contrast, mRNA levels fluctuate in sympathy with the synthetic activity of the cell.

That nucleic acids occur ubiquitously in prokaryotic and eukaryotic cells would appear to make them suitable markers for cellular material in general. However, because of their presence in most natural feeds, they would appear to be unsuitable as specific, endogenous, markers of microbial nitrogen in ruminant digesta. The marker attribute of importance here is intimate and specific association with the material to be marked (Kotb and Luckey, 1972). Microbial nucleic acids obviously fulfil the 'intimate' criterion, and may be considered to satisfy the 'specific' requirement either if a synthetic diet containing no nucleic acid is fed or if all dietary nucleic acid is degraded in the gastro-intestinal tract anterior to the point of sampling.

It is generally considered that dietary DNA and RNA are rapidly degraded in the rumen (Smith, 1969; Smith and McAllan, 1970;

McAllan and Smith, 1972; Razzaque and Topps, 1972). It has also been demonstrated that even if no ruminal degradation occurred, dietary DNA could only account for 5 - 10% of the DNA in the rumen (Wolstrup and Jensen, 1978). Smith and McAllan (1970) administered reagent RNA and DNA intraruminally to calves to give concentrations four times those resulting from feeding natural diets. The polymers were destroyed within one hour, being transiently replaced by nucleotides and purine and pyrimidine bases. These authors noted that the natural diets they used contained enough DNA and RNA to account for 20 and 50% respectively of these nucleic acids in rumen fluid. However, the rapid degradation of reagent nucleic acids (which they observed in in vitro studies), and the fact that the RNA:DNA ratio in rumen fluid was not influenced by the levels of these nucleic acids in the diet, led McAllan and Smith (1970) to conclude that dietary nucleic acids were almost completely degraded in the rumen.

Razzaque and Topps (1972) added varying levels of RNA or DNA to a diet with a high starch content which was fed to two lambs fitted with abomasal cannulas. Large variations in dietary RNA and DNA intake were therefore achieved, but faecal excretion and the content of these nucleic acids in the abomasal digesta remained approximately constant. The authors concluded that these results supported earlier findings on degradability of RNA and DNA in the reticulo-rumen.

McAllan and Smith (1974) determined the microbial N contribution to total non-ammonia N reaching the duodenum of a defaunated calf, using RNA and DAPA as endogenous markers. They considered that the good agreement obtained between the two techniques provided

circumstantial evidence that any dietary RNA was completely degraded in the rumen.

In their study of a range of microbial markers, Ling and Buttery (1978) used RNA along with DAPA and radio-labelled sulphur amino acids (see Section 2.3.2.2.1 below). With one diet, in which the majority of the nitrogen was supplied as urea, the proportion of microbial N in the duodenal total N was greater than unity when RNA was used as the marker. It was considered that the analytical technique used to detect RNA in digesta was accurate, and the authors were concerned that some dietary RNA, in common with other dietary nutrients, escaped ruminal degradation and was present in duodenal digesta.

A variety of techniques have been employed to extract, purify and measure nucleic acids from plant (Thomas and Sherrat, 1956; Kupila, Bryan and Stern, 1961; Zscheile and Murray, 1963; Guinn, 1966), animal (Logan, Mannell and Rossiter, 1952) and microbial (Crestfield, Smith and Allen, 1955) material. Such techniques have been adapted to determine nucleic acid concentrations in digesta and microbial samples from ruminants (McDonald, 1954; McAllan and Smith, 1969; Ling and Buttery, 1976, 1978).

The most commonly used substances for extracting nucleic acids from biological materials are acid (normally perchloric acid), alkali (normally potassium hydroxide), neutral salt (normally sodium chloride), phenol and detergents (Guinn, 1966). In experiments in which potassium hydroxide has been used, nucleic acids have been extracted in the presence of large amounts of material which interfered with subsequent attempts to measure them (Guinn, 1966; McAllan and Smith, 1969; Czerkawski, 1976). McAllan and

Smith (1969) and Czerkawski (1976), who used similar methods, measured RNA and DNA levels in samples of ruminant digesta. Extraction and hydrolysis with potassium hydroxide yielded an acid soluble fraction whose ultraviolet (u v) absorption spectrum was dissimilar to those obtained with pure nucleotide solutions (McAllan and Smith, 1969). These workers therefore had to purify the digesta by passing it through an ion-exchange resin column. The introduction of additional steps in the analytical procedure obviously reduced the rapidity with which analyses could be performed.

Crestfield (1955) described a procedure by which undegraded RNA could be extracted from bakers' yeast by the use of hot sodium-dodecyl sulphate. Subsequent precipitation with cold sodium chloride yielded a preparation which was pure, and which contained 60 - 70% of the original yeast RNA. When using a buffered mixture of phenol and sodium lauryl sulphate, Guinn (1966) similarly found that only a portion of the RNA was extracted from freeze-dried plant material. Additionally, he observed that the extracted material contained much interfering carbohydrate, protein and phosphate.

Hot sodium chloride has been used successfully by several workers for the quantitative extraction of nucleic acids from plant material (Thomas and Sherratt, 1956; Kupila et al, 1961; Zscheile and Murray, 1963; Guinn, 1966). The techniques of these workers share the use of sodium chloride solution at 100^{o}C as the extractant. Several extractions were performed on each sample by Zscheile and Murray (1963) and by Guinn (1966) whereas Thomas and Sherratt (1956) and Kupila et al (1961) considered a single aliqout of the extracting solution to be adequate. Guinn (1966)

found that the u v spectra of nucelotide solutions, prepared by acid hydrolysis of the nucleic acids precipitated from the extraction solution with cold trichloroacetic acid, were free from interfering material. At wavelengths above 220 nm they were very similar to those obtained from reagent RNA or sperm DNA.

In an attempt to improve upon the speed and simplicity of the techniques then available (McAllan and Smith, 1969), Ling and Buttery (1976) adapted the hot sodium chloride extraction procedure of Guinn (1966) and used it to determine the nucleic acid contents of mixed rumen bacteria and duodenal digesta. In a subsequent report of their work (Ling and Buttery, 1978), they stated that the technique was rapid (20 samples analysed d^{-1}) and accurate (the u v absorption spectra of the extracted nucleotides were 'fairly free' from interfering substances).

Extracted nucelic acids may be detected, following hydrolysis, either by colorimetric estimation of the ribose and deoxyribose (Davidson, 1969)or by spectrophotometric estimation of nucleotide levels (Zscheile and Murray, 1963; Guinn, 1966; Ling and Buttery, 1976, 1978). RNA and DNA may be differentiated by the specific colour reactions of ribose and deoxyribose with orcinol and diphenylamine respectively (Guinn, 1966; Davidson, 1969). McAllan and Smith (1969) use u v absorption to measure RNA and the diphenylamine test to measure deoxyribose from DNA. A range of u v wavelengths, from 230 -340 nm, have been used alone, and in various combinations, to estimate the nucleic acid (or nucleotide) content of solutions (Zscheile and Murray, 1963). The customary use of 260 nm alone (McAllan and Smith, 1969) is not the best choice, 268.7 nm being the most suitable single wavelength (Logan et al,

1952; Zscheile and Murray, 1963; Davidson, 1969). The combination of absorptions at 260 and 280 nm has also been suggested as a suitable indicator of total nucleic acid content (Ling and Buttery, 1976; 1978).

As well as being readily detectable, a marker must bear a fixed, or measurable, relationship to the material it is to mark (Kotb and Luckey, 1972). For nucleic acids, it is vital that the ratio of total nucleic acid, RNA or DNA:total microbial N is known if the content of the marker in a digesta sample is to be converted into a value for microbial N.

The ratio is most frequently expressed as nucleic acid N (or RNA N or DNA N): microbial N, using values of 140 and 148 g N kg^{-1} for RNA and DNA compositions respectively (McAllan and Smith, 1969).

In general, the overall ratio has been found to be relatively invariable for mixed rumen microbial populations (Ellis and Pfander, 1965; Smith, McAllan and Hill, 1968; Smith, 1969; Smith and McAllan, 1970; Smith et al, 1978). Since the amount of DNA within a cell is relatively constant whereas total RNA levels vary with the synthetic activity of the cell (see above), several workers have found that RNA N:total microbial N is a more constant ratio, and therefore a better predictor of microbial N levels, than is DNA N:total microbial N (Smith, 1969; Czerkawski, 1976; Ling and Buttery, 1978). Ling and Buttery (1978) observed coefficients of variation of 37.5% and 9.9% respectively for DNA N:total N and RNA N:total N ratios in bacterial isolates. Smith and McAllan (1974) demonstrated similar nucleic acid N:total microbial N ratios for rumen bacterial isolates from cows and sheep. Bacteria from younger cattle had ratios which were influenced by the environment in which they were reared,

proximity to adult ruminants affecting their gut microflora. These workers also observed that bacterial composition varied with time after feeding, emphasising the need for continuous feeding or for frequent sampling over a complete feeding cycle.

There have been several reports of the use of nucleic acids as indices of the microbial N levels in ruminant digesta and hence of the degradability of dietary N. The technique has been used at the National Institute for Research in Dairying (Smith and McAllan, 1973; McAllan and Smith, 1974; Smith et al, 1978), at the University of Nottingham (Ling and Buttery, 1976, 1978) and at the Grassland Research Institute (Beever and Siddons, 1978).

When offering a series of roughage and concentrate diets to cannulated calves, Smith and McAllan (1973) observed that 5.5 h after the food was given, RNA N levels in rumen liquor had risen by 50 - 100%. In duodenal digesta, the RNA N:non ammonia N ratio was lower than had been observed in rumen liquor. This was interpreted as resulting from the secretion of endogenous N compounds into the gut. The authors estimated that between 47 and 79% of the non-ammonia N in duodenal digesta was microbial in origin. Smith et al (1978) estimated the amounts of microbial N entering the duodenum of cattle offered mixed flaked maize and roughage (hay or straw) diets supplemented with decorticated groundnut meal, fishmeal, heated soyabean meal, raw soyabean meal or dried grass. DAPA, RNA and ^{32}P radiolabelled nucleic acids (see Section 2.3.2.2.2 below) were all used as indices of the microbial content of digesta. In general, estimates of the microbial content of digesta, based on RNA N levels, were 118% of those derived from ^{32}P-labelled RNA nucleotides. This was thought to be caused by incomplete degradation of

dietary RNA, and the use of an appropriate correction factor meant that RNA-derived values agreed with those from ^{32}P labelling. Mean degradabilities of food N, derived from the average microbial N levels from adjusted RNA and DNA measurements, ranged from 0.57 (maize/hay diet) to 0.84 (maize/straw/raw soyabean diet) although no between-diet differences were significant ($P > 0.05$).

Ling and Buttery (1976, 1978) described a series of experiments which compared several techniques for measuring microbial N in the digesta of cannulated wethers. They also fed a range of diets based on barley grain and straw, with approximately 40% of the nitrogen derived from fishmeal, soyabean meal or urea. When compared with radiolabelled (^{35}S) amino acids (see Section 2.3.2.2.1 below) (Ling and Buttery, 1976) RNA N gave higher values for the proportion of microbial N:total N in duodenal digesta. Both techniques ranked the diets in the same order (urea > soyabean meal > fishmeal) in terms of the proportion of microbial N in the digesta sample. When tested in conjunction with DAPA (Ling and Buttery, 1978), the same trends were apparent and all three techniques provided the same ranking of the diets. The RNA method yielded estimates of microbial N which were higher, in absolute terms, than those for ^{35}S and DAPA.

Beever and Siddons (1978), considered four different methods for measuring microbial protein synthesis in the rumen. RNA, used as a microbial marker, gave higher estimates than DAPA, ^{35}S or isotopically labelled N (^{15}N). They pointed out that, as with all such studies, it was impossible to comment unequivocally on the absolute accuracy of their techniques. However, use of ^{15}N gave the set values with the lowest coefficient of variation.

2.3.2.1.4 Phosphatidyl choline

Phosphatidyl choline has been suggested as a specific endogenous marker for rumen protozoa (John and Ulyatt, 1979). The compound is found widely in protozoa, including those of the rumen, occurs only rarely in bacteria and is not secreted into the gastrointestinal tract anterior to the bile duct in significant amounts (John and Ulyatt, 1979). Very low levels of the compound (1 μg phosphatidyl choline P g^{-1} DM) were detected in a lucerne hay diet fed to cannulated wethers, and all the material was rapidly degraded in the rumen. Phosphatidyl choline could not be detected in isolated rumen bacteria. The ratio of phosphatidyl choline P: total N in mixed rumen protozoa was not influenced by intake level or feeding frequency and the authors therefore considered the material a suitable marker. Using the technique, John and Ulyatt (1979) observed between 0.12 - 0.23 of total microbial N to be protozoal in origin.

2.3.2.2. Exogenous microbial markers

As an alternative to the use of endogenous markers, the microbial component of duodenal digesta may be identified if it has been labelled by the introduction of an exogenous marker which has been incorporated in some microbial molecule (Ling and Buttery, 1978). The most frequently used techniques involve radio-labelling of sulphur-containing microbial amino acids (Harrison, Beever and Thomson, 1972; Ling and Buttery, 1978) or of microbial nucleic acids (Smith et al, 1978). Alternatively, microbial nitrogen as a whole may be marked with non-radioactive, isotopic nitrogen (Smith, 1975; Buttery, 1977; Lazenby, 1980).

2.3.2.2.1 ^{35}S-labelled microbial amino acids

Work at the Grassland Research Institute (Harrison et al, 1972; Beever, Harrison and Thomson, 1972; Beever, Harrison, Thomson Cammell and Osbourne, 1974) established the use of ^{35}S-labelled amino acids as indices of microbial nitrogen in duodenal digesta. The technique used ^{35}S-labelled sodium sulphate ($Na_2{}^{35}SO_4$) infused intraruminally at rates of 3.0 μCi h^{-1} (Beever et al, 1974) to 10 μCi h^{-1} (Harrison et al, 1972), as a source of ^{35}S which was subsequently incorporated in microbial, sulphur containing, amino acids. Once equilibrium had been established, radioactive counts were obtained for microbial and digesta methionine (N_m and N_d respectively). Similarly, the concentrations of methionine were determined in isolated rumen microbes (C_m) and in duodenal digesta (C_d). The resultant specific activities ($N_m/C_m = M$; $N_d/C_d = D$) were then compared. An M:D ratio of 1 was expected if all duodenal methionine was microbial in origin. In a series of four experiments, in which foods containing no protein were offered to cannulated wethers, Beever et al (1974) observed M:D ratios of 0.96, 1.06, 1.02 and 1.01 . The overall mean of these observations did not deviate significantly from 1.0. Dilution of digesta methionine with methionine from undegraded feed protein would decrease the specific activity of that digesta methionine and thus increase the M:D ratio. In their studies, Beever et al (1974) demonstrated that this occurred when ground sanfoin was added to digesta which had been collected from a sheep fed a protein free diet. Beever et al (1972) and Beever et al (1974) demonstrated that the M:D ratio, and hence the estimated proportions of protein of dietary and microbial origin, showed marked diurnal variation. However,

when sampling at 2 h intervals, the mean M:D ratio of the 12 samples gave a microbial:food protein estimate very close to that derived from the M:D ratio from a bulked 24 h total collection of digesta.

It is important to appreciate that the partitioning of digesta protein into microbial and residual food fractions will only be accurate if the methionine concentration in residual food protein in duodenal digesta is the same as in the original food (Beever et al, 1974). The specific activity of digesta methionine could be reduced by 'contamination' with endogenous methionine from proteinaceous secretions into the tract. The observation that, when protein free diets are fed, the M:D ratio does not deviate from unity (Beever et al, 1974) provides circumstantial evidence that the effect is not important in vivo.

Beever and Siddons (1978) used ^{35}S-labelled microbial amino acids in a comparative study of techniques for measuring microbial protein synthesis in ruminants. This technique gave the lowest estimate of protein synthesis, and its coefficient of variation was higher than that for ^{15}N-labelling. In a similar experiment, Ling and Buttery (1978) compared ^{35}S-labelled amino acids with RNA and DAPA as estimators of the degradability of various food proteins. Each method ranked the diets in the same order, but the absolute proportions of microbial protein in duodenal digesta were highest for RNA and lowest for DAPA, with ^{35}S-derived values intermediate. The authors considered that the ^{35}S figures were the most accurate.

2.3.2.2.2 ^{32}P-labelled microbial nucleic acids

This technique has been used at the National Institute for Research in Dairying as an extension of the use of RNA or microbial

nucleic acids as endogenous microbial markers (Smith, Lewis and McAllan, 1976; Smith et al, 1978). The technique involves the intraruminal administration of a radioactively labelled inorganic phosphate solution. Smith et al (1978) gave 0.25 m Ci ^{32}P as labelled sodium phosphate ($Na_2{}^{32}PO_4$) twice daily to Friesian steers. This inorganic phosphate was incorporated into rumen bacteria, and, when equilibrium was established after 4 d, the specific activity of ^{32}P-labelled RNA nucleotides, extracted using the technique of McAllan and Smith (1969), was determined. For a range of diets, estimates of the microbial contribution to total duodenal N were smaller when ^{32}P-labelled nucleotides were used as the microbial marker instead of unlabelled RNA. Smith et al (1974) were more confident of the accuracy of their radio-labelled method and therefore corrected RNA values to agree with it. They also observed that the specific activity of RNA nucleotides in bacterial isolates was higher than that of RNA nucleotides in duodenal digesta. This demonstrated that not all of the RNA nucleotides in duodenal digesta were bacterial in origin, but could not show whether the contaminating material was microbial (i e protozoal), dietary or endogenous.

2.3.2.2.3 ^{15}N-labelled microbial nitrogen

The use of ^{15}N, a non-radioactive isotope, has been one of the most important tracer techniques for the study of intermediary nitrogen metabolism in ruminants (Smith, 1975; Lazenby, 1978). The use of ^{15}N-labelled bacterial and protozoal N to measure compartmentalisation of the N in digesta has been reported (Pilgrim, Gray, Weller and Belling, 1970; Mathison and Milligan, 1971), but the technique involves such expensive and complex analytical apparatus (^{15}N is measure in a mass spectrometer) that its

applications have been limited (Ling and Buttery, 1978).

Mathison and Milligan (1971) used a continuous infusion of ^{15}N-labelled ammonium chloride ($^{15}NH_4Cl$) to label all the products of nitrogen metabolism, in sheep fed hay or barley diets. The level of ^{15}N in microbial isolates from ruminal and abomasal digesta samples allowed estimations to be made of the contribution of microbial N to the total N flow from the rumen.

2.3.2.3 Constrained optimisation of the duodenal amino acid profile

A novel method of determining the relative levels of microbial, endogenous and residual food proteins in ruminant duodenal digesta has been developed at the University College of North Wales (Evans, Axford and Offer, 1975; Offer, Axford and Evans, 1978). The technique assumes that the amino acid profile observed in duodenal digesta must be the weighted sum of the profiles contributing to it. Thus, given the amino acid profiles of dietary, microbial and endogenous proteins, it is possible to test statistically the possible combinations of these compounds to find the composite profile which gives the best fit with that observed in duodenal digesta. The success of the technique depends on (i) accurate amino acid profiles for all the factors, (ii) adequate computing power to allow the profile combinations to be rapidly tested and (iii) suitable constraints being placed on the levels which each of the three constituent fractions can take. The whole digesta profile can be accurately determined, but the residual dietary protein must be assumed to be identical to the whole diet which was fed. The microbial profile must be determined on a sample of microbes taken from the rumen. Since no information was available on the proteins constituting the 'endogenous' factor, Evans

et al (1975) used the amino acid profile of bovine pepsin. The general constraints applied to the process of fitting the amino acid profiles were that no dietary constituent of digesta was allowed to exceed the intake of that constituent, and no constituent was allowed to make a negative contribution to the final profile (Offer et al, 1978).

2.3.3 Measurement of endogenous nitrogen in intestinal digesta

In contrast with measurement of microbial N, measurement of the contribution of nitrogen-containing compounds of endogenous origin to the flow of total N into the ruminant small intestine, has attracted little research effort (Smith, 1975; Buttery, 1977; Ling and Buttery, 1978). Ideally, consideration of this fraction is necessary if the degradability of dietary N is to be assessed (see Section 2.3.1). Frequently, however, endogenous N is ignored or is assigned an arbitrary value (Harrop, 1974; Smith et al, 1978; ARC, 1980). If no allowance is made for the endogenous component of duodenal N, true values of the proportion of dietary N degraded in the reticulo-rumen are underestimated (ARC, 1980). The extent and importance of this underestimation will obviously depend on the relative size of the endogenous N fraction.

Smith and McAllan (1973), when feeding five different diets to cannulated calves, estimated that 14 - 26% of duodenal non-ammonia N was endogenous in origin. These estimates were derived from observations of non-ammonia N:RNA N ratios in rumen and duodenal digesta samples. The ratio was higher in duodenal digesta and this was interpreted as being due to the secretion of nitrogenous compounds into the gastrointestinal tract between the rumen and the duodenum. Ling and Buttery (1978) observed that a total of 1.71 g non-microbial N d^{-1} was present at the duodenum when a diet, whose only nitrogen sources

were barley and urea, was fed to sheep. The authors considered that virtually all of this N could be attributed to endogenous compounds because of the high degradability of the barley's N.

Provided that the site of cannulation is anterior to the common bile and pancreatic duct (Thomas, 1978), the main source of endogenous N in samples of duodenal or abomasal digesta is pepsinogen, secreted by the fundic tubular glands in the abomasum (Van Bruchem and Van't Klooster, 1980). Some observations have suggested that these secretions are quantitatively unimportant. The technique of Harrison et al (1972), which uses ^{35}S to label the sulphur-containing amino acids of rumen microbes (see Section 2.3.2.2.1), also allows the presence of unlabelled endogenous N to be detected if a food containing no protein is fed. Using this technique Beever et al (1974) fed diets, containing urea as the sole source of nitrogen, to cannulated sheep. The ratio of the specific activities of microbial methionine to duodenal digesta methionine was never significantly different from unity. As has been stated previously, this demonstrated that no residual dietary protein was present in the digesta sample. However, it also indicated that no unlabelled N, and by implication no endogenous N, was diluting the labelled microbial N in duodenal digesta.

2.3.4 Alternative methods of assessing the degradability of dietary nitrogen

From the expression which describes the proportion of dietary N which is degraded in the rumen:

$$1 - \frac{\text{(duodenal N - duodenal microbial N - duodenal endogenous N}}{\text{dietary N}}$$

(ARC, 1980), it can be seen that dietary N degradability can only be measured in animals which have been cannulated at, or near, the

duodenum. Additionally, it may only be measured if the endogenous and, more importantly, microbial components of duodenal N can be determined by the techniques described above (see Section 2.3.2, 2.3.3). As a result, attempts have been made to relate true degradability to more readily measurable parameters such as protein solubility *in vitro* (Wohlt, Sniffen and Hoover, 1973; Crawford, Hoover, Sniffen and Crooker, 1978; Chamberlain and Thomas, 1979) and rate of N disappearance from intra-ruminally incubated artificial fibre bags (Mathers, Horton and Miller, 1977; Ørskov and Mehrez, 1977; Smith and Mohamed, 1977; Siddons, 1978). It is vital that the validity of such techniques be assessed by investigating their correlation with degradability measured with cannulated animals (Chamberlain and Thomas, 1979; Mahadevan, Erfle and Sauer, 1980).

Crawford *et al* (1978) observed the solubilities of the N in 28 foods in three solvents (10% Wise-Burroughs mineral buffer, 0.15 M sodium chloride and autoclaved rumen fluid), and attempted to correlate these with the rates of disappearance of N after 2 h inter-ruminal incubation in artificial fibre (Dacron) bags. The correlations were highest for silages and hays incubated in the Wise-Burroughs buffer.

Chamberlain and Thomas (1979) examined three laboratory tests designed to assess the susceptibility of feed proteins to microbial breakdown in the rumen. The tests were (i) N solubility after 6.5 h incubation in buffered artificial saliva, (ii) non-precipitable N produced following 6.5 h incubation in buffered artificial saliva containing bacterial protease and (iii) ammonia N production following 6.5 h anaerobic incubation with strained rumen fluid. The three methods gave different estimates for each of the four foodstuffs tested (casein, groundnut meal, soyabean meal and white fishmeal).

In some instances (casein degradability measured by tests (i) and (ii), white fishmeal degradability measured by test (iii)) the degradabilities fell within the range reported for degradabilities measured in vivo. As initially tested, the rumen fluid incubation test was ineffective because of concomitant fixation of evolved ammonia due to the fermentation of carbohydrate in the feeds.

Mahadevan et al (1980) demonstrated that simple measures of protein solubility were unlikely to provide reliable indices of susceptibility to ruminal degradation. Soluble and insoluble proteins were incubated in a phosphate buffer solution containing semi-purified protease extracted from the rumen bacterium Bacteroides amylophilus. Soluble proteins such as serum albumin and ribonuclease A were resistant to hydrolysis, soluble and insoluble proteins in soyabean meal were hydrolysed at identical rates and soluble proteins from different foods were hydrolysed at different rates.

The artifical fibre bag technique (Mehrez and Ørskov, 1977) has been used to determine the rate and extent of degradation of dietary protein (Ørskov and Mehrez, 1977). Wethers, fitted with large diameter rumen cannulas, were maintained on a diet of good quality hay. Samples of dry feeds were ground, and fresh or wet materials minced, before being placed in artifical fibre bags which were incubated in the rumen. Samples of any given food were removed after a series of incubation times to determine the rate of disappearance of protein and dry matter. These authors considered that the protein degradation occurring under normal feeding conditions had taken place when 90% of the digestible dry matter had disappeared from the bag. Using these criteria, values of 0.82, 0.78 and 0.60 were obtained for the extent of protein degradation in timothy, ryegrass/timothy and maize silages respectively.

Smith and Mohamed (1977) used a similar technique, and demonstrated qualitative changes in the protein remaining in the artificial fibre bags as the incubation period increased. In particular, available methionine was lost more readily than was total methionine.

Mathers et al (1977) found that the disappearance of N after 4 - 6 h intra-ruminal incubation gave degradability estimates which agreed most closely with estimates of degradability measured in vivo. Dry matter disappearance at this time varied from 15 - 80%. It is of interest that Mahadevan et al (1980) (see above) reported a lag period of approximately 6 h before microbial protease started to hydrolyse proteins in vitro.

Mathers et al (1979) were also concerned with the nutritive value of that portion of dietary protein which escaped ruminal degradation. They therefore compared the growth rates of weanling rats fed semi-purified diets in which all of the N was derived from fishmeal, soyabean meal or residues from these feeds which had been incubated in polyester bags in the rumens of sheep for 6 h. The nutritive value of fishmeal was unaltered, but the growth rate and efficiency of protein utilisation were lower for the degraded soyabean residue than for the original material. The overall digestibility of N was the same for both soyabean rations and the authors concluded that the different growth was attributable to a poorer amino acid balance in the degraded soyabean meal.

Siddons (1978) showed that the diet of the 'host' animal and hence the intraruminal environment, could influence the rate of loss of material from incubated artifical bags. Four diets (flaked maize, rolled barley, timothy and lucerne), when fed to sheep gave rise to different disappearance rates for a quantity of casein given intra-

ruminally. The highest and lowest rates were obtained with maize and timothy respectively. Confusingly, the disappearance of N from soyabean meal, groundnut meal and fishmeal, incubated in polyester bags, was higher when timothy, barley or lucerne, as opposed to flaked maize, was fed.

Morgan, Edwards and McDonald (1980b), in the course of a series of experiments on the intake and metabolism of fresh and wilted rye-grass silages, observed the patterns of disappearance of N from samples of fresh and wilted silages and of a pelleted complete ruminant diet incubated in nylon bags in the rumens of cannulated steers. Bags were incubated in the rumen of animals being fed the diet whose degradation was being measured. Proportional disappearance of N was slightly greater for fresh than for wilted silages at all incubation times. However, both silages had lost 0.7 to 0.8 of their original N following 1 h intra-ruminal incubation whereas only 0.15 of the N in the pelleted diet had been lost in the same period.

2.4 THE DIGESTION AND METABOLISM OF SILAGE NITROGEN BY RUMINANTS

2.4.1 Introduction

The feature of digestion in ruminants, which sets that suborder of animals apart from other herbivores, is the extensive degradation of dietary nutrients by the microbial population of the reticulo-rumen (McDonald et al, 1973; Armstrong, 1980; see Section 2.3). The ability which these symbiotic bacteria confer on their ruminant hosts to utilise plant carbohydrates, in particular those which are resistant to digestion by the enzymes of higher animals (e g cellulose and hemicelluloses), is unsurpassed amongst mammals (McDonald et al, 1973; Koller, Hintz, Robertson and Van Soest, 1978). Bacteria are also responsible for the extensive degradation of dietary protein and recycled urea, which occurs in the reticulo-rumen and which has a pronounced effect on the profile of nitrogenous compounds which is presented at the small intestine for subsequent digestion, absorption and metabolism by the host animal (McDonald et al, 1973; Smith, 1969; Smith, 1975; Mathers et al, 1979; Armstrong, 1980).

2.4.2 Digestion of nitrogenous constituents of food in the rumen

The principles of microbial intervention, which may markedly alter the dietary nitrogen components before they reach the ruminant small intestine, have been appreciated for a considerable time (Smith, 1969), and rationing systems based on this framework have been proposed (Roy, Balch, Miller, Ørskov and Smith, 1977; ARC, 1980). The systems are, essentially, simple. The nitrogenous compounds which enter the rumen are the protein and non-protein N contained in the diet, together with recycled N which occurs as urea in the copious quantities of saliva characteristically produced by the ruminant (Smith, 1969; Armstrong, 1973; Armstrong, 1980). Rumen bacteria degrade these

compounds producing peptides, free amino acids and ammonia in the case of dietary protein (Smith, 1969; Armstrong, 1973; Ørskov and Mehrez, 1977; ARC, 1980; Armstrong, 1980; Beever, 1980) whereas the non-protein N in foodstuffs is generally considered to be rapidly degraded to ammonia (Smith, 1969; Beever, 1980). These products of the degradation of dietary N then provide the substrate which, with the energy provided by the fermentation of dietary carbohdyrate, is assimilated into microbial protein. The nitrogenous nutrients which then reach the small intestine, following acid/pepsin digestion in the abomasum, are an amalgam of peptides and amino acids of microbial origin together with those from the diet which were, for some reason, not degraded by the rumen bacteria (Smith, 1969; Armstrong, 1973; Smith, 1975; Mathers et al 1979; ARC, 1980; Armstrong, 1980: Beever,1980). Endogenous N compounds, from sloughed epithelial cells and secretions into the intestinal tract, also contribute to the nutrient profile reaching the duodenum (ARC, 1980: Beever, 1980). This process of ruminal transformation of dietary N has been illustrated schematically by several authors (McDonald et al, 1973; Beever, 1980) and such a diagram is presented below (Fig 2.4.1).

It can be appreciated from Fig 2.4.1 that the balance between dietary and microbial amino acids will determine the biological value of the amino acid profile in the duodenum. With some diets, in which protein has been rendered resistant to microbial degradation by heat or chemical treatment, amino acids from undegraded dietary protein may be the more important component (ARC, 1980; Thomas et al, 1980). Usually,however, the constancy of the amino acid profile at the duodenum and the fact that this profile is insensitive to changes in the amino acid composition of the diet, indicate the overwhelming importance of the degradation of dietary N and subsequent synthesis

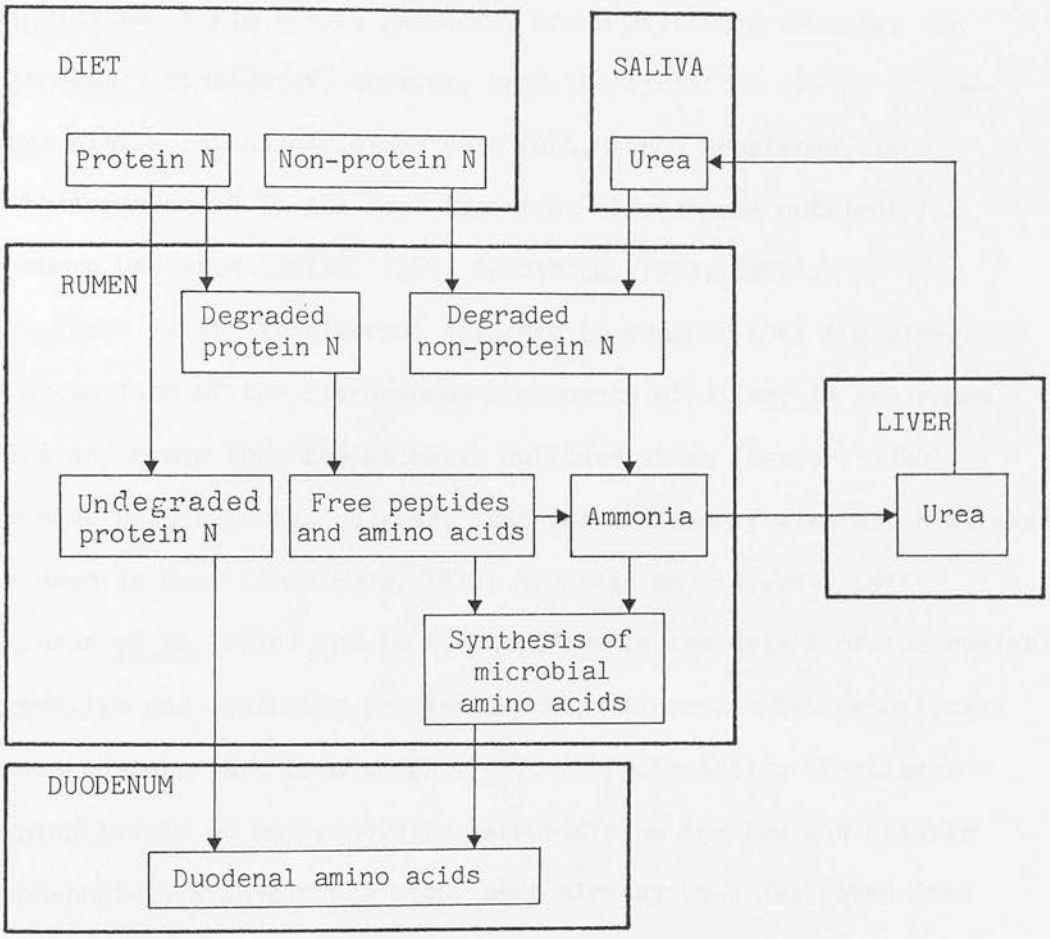

Fig 2.4.1 Digestion and metabolism of nitrogenous compounds in the rumen

of microbial amino acids, to the protein nutrition of the host animal (Chamberlain and Thomas, 1979; Gill et al, 1979). Thus the degradation of protein and non-protein N in the rumen and efficiency with which microbial protein is subsequently synthesised (i e the provision of substrates and the subsequent utilisation of those substrates by the rumen microbes) are likely to be the factors of greatest importance in determining the nutritional value of the protein in a ruminant's diet (Armstrong, 1973; Beever, 1980).

The nitrogenous substrates used by the rumen bacteria are, as indicated in Fig 2.4.1, peptides, amino acids and ammonia. It is generally considered, however, that the first two groups of compounds play a relatively minor role (ARC, 1980; Armstrong, 1980) and that ammonia N is the most important nitrogenous nutrient for the rumen bacteria (Smith, 1969; Armstrong, 1973, 1980).

There is no experimental evidence to suggest that the digestion and metabolism of the nitrogenous components of silage in the rumen follow any other than the pathways outlined above (Beever, 1980). There have been reports, however, that the efficiency with which silage N is used is poor (Armstrong, 1973; McDonald and Edwards, 1976; Wilkinson et al, 1976) and it is therefore in the detail of the ruminal degradative and synthetic processes that differences between silages and other diets have been sought. The characteristics of silage, in which levels of water-soluble carbohydrates are low and soluble or non-protein N levels are high, have already been described (see Section 2.1). A resulting imbalance in the provision of energy and nitrogen for bacterial protein synthesis may therefore occur as the soluble N compounds are rapidly degraded to ammonia in the rumen (Smith, 1969; Armstrong, 1980). In the following sections, the related aspects of dietary N degradation, ruminal ammonia levels, the synthesis of microbial protein and the supply of amino N to the small intestine, will be considered in greater detail.

2.4.3 Degradation of dietary nitrogen

The degradability of dietary N may be measured using cannulated animals (see Section 2.3.2) or it may be estimated from measurements of solubility made in vivo (see Section 2.3.4) (ARC, 1980). Although correlations between solubility and degradability are sometimes poor

(Mahadevan et al, 1980), grass silages, for which no pre-ensiling additive treatment has been used, have been categorised as having highly degradable N (ARC, 1980; Beever, 1980). Whether this is true of the small amount of residual protein in silage is, however, unclear (Thomas et al, 1980; Beever, 1980).

Ørskov and Mehrez (1977) estimated the degradability of the nitrogen in several untreated silages using an artificial fibre bag technique. That of silages made from timothy (Phleum pratense) and timothy/ryegrass mixtures was readily degraded (mean values being 0.82 and 0.78 respectively). Other common foodstuffs had similarly high degradabilities (swede roots, 0.89; barley, 0.73 - 0.80; wheat, 0.72; dried grass, 0.70) but the N in maize and maize silage was less degradable (0.55 and 0.60 respectively).

Crawford et al (1978) found that a higher correlation, between solubility and intra-ruminal degradation, was obtained for silage than for hay N. Morgan et al (1980b) observed that the loss of nitrogen from silages incubated in artificial fibre bags in the rumens of fistulated steers was very rapid, only 0.2 - 0.3 of the original being left after 1 h. In this experiment, wilting did not markedly alter the protein N content of the resulting silage nor, consequently, the rate of N loss.

Beever (1980) commented that although the degradable N content of untreated silages need not be any higher than that of the fresh grass from which the silage was made, the degradable carbohydrate content of the latter was higher, resulting in a more 'beneficial' available carbohydrate:N ratio when the grass was digested by ruminants.

Where the effects of pre-ensiling additive treatments on the degradability of silage N have been reported, significant results have tended to show a reduction in the parameter (Beever, 1980). The magnitude and consistency of these effects depends on the particular additive used, and the most conclusive results have been obtained with formaldehyde (Barry, 1976; Siddons and Evans, 1978). Initially, formaldehyde was used to reduce the ruminal degradation of casein being fed in semi-purified diets to ruminants (Armstrong, 1973). Barry (1976) explained the chemistry of the reaction between formaldehyde and herbage protein. The reaction is a two stage process, the initial step involving the rapid formation of a methylol group (Barry, 1976; Ohshima and McDonald, 1978), followed by condensation reactions which take place at a slower rate and which lead to the formation of stable methylene cross-linkages between protein chains (Barry, 1976). These stabilising linkages are susceptible to hydrolysis in the acid conditions of the abomasum (Barry, 1976). For some amino acids however (the effect has been demonstrated for lysine, threonine and the sulphur-containing amino acids methionine, cystine and cysteine) the reversibility of the reaction with formaldehyde is incomplete and availability at the small intestine may be unaltered or even reduced by pre-ensiling treatment.

Beever et al (1977) demonstrated reduced ruminal degradation of dietary N for silage made from perennial ryegrass (cv S24) treated with 6.4 l formalin t^{-1} at ensiling, compared with an untreated control. Using sheep fitted with re-entrant duodenal cannulas, they found that the proportion of amino acids of microbial origin in duodenal digesta decreased from 0.71 to 0.18 in response to the additive treatment. Total amino acid supply to the small intestine

was also 14% higher for the treated material.

Siddons and Evans (1978) investigated one of the potential problems of formaldehyde additives - the reduction of the degradability of dietary N to the extent that the supply of degradable N is inadequate for maximum microbial growth. When applied at a rate of 35 g kg^{-1} herbage protein, formaldehyde produced a silage which increased the supply of amino N to the small intestine when compared with an untreated control. The workers had considered that the formaldehyde-treated silage would supply insufficient rumen degradable N to satisfy the needs of the rumen microbes. However, the microbial N flow to the duodenum was the same for the treated and untreated silages and, in the case of the former, was unresponsive to the supplementation of the diet with urea.

Beever (1980) reported that formaldehyde, applied to ryegrass at a rate of 60 g kg^{-1} crude protein as a pre-ensiling treatment, encouraged an increase of 33% in the total flow of amino acids to the duodenum of sheep while at the same time reducing the ruminal degradability of dietary N from 0.85 to 0.22.

Gill et al (1979) investigated the effects of a combination of formalin (4.8 l t^{-1}) and formic acid (1.0 l t^{-1}) applied together to a mixture of perennial ryegrass and white clover (Trifolium repens) prior to ensiling. Compared with a similar untreated silage, the treated material had a far higher protein N content (806 vs 472 g kg^{-1} total N) although low water-soluble carbohydrate levels indicated that extensive fermentation had occurred in both silos. The degradability of dietary N was not measured directly, but the fact that the proportion of dietary N apparently digested between the mouth and the duodenum was much higher for the untreated than for the

treated diet was taken as evidence that the additives had rendered the N in the latter less susceptible to ruminal degradation. The protection of dietary protein was not complete, however, and differences in amino acid composition which could be detected between the diets were not apparent in the duodenal digesta.

Thomas et al (1980) prepared three silages from perennial ryegrass harvested at different times during the growing season, all of which were treated with formic acid before ensiling. For all silages, the contribution of bacterial N to total duodenal N, and hence the estimate of the degradability of dietary N, was relatively low. Further evidence that rates of protein degradation in the rumen were low was obtained from the amino acid profiles of duodenal contents, which were directly influenced by the profiles of the diet being fed.

2.4.4 Ruminal ammonia levels

Ammonia is a product of microbial digestion of nitrogenous constituents of the diet and the end product of the hydrolysis of urea (Chalmers, Jaffray and White, 1971; see Fig 2.4.1). The terminology of this subject area can be confusing with ammonia, ammonia N, ammonium, NH_4^+, NH_3 and NH_3N all being used with more or less precision by different authors. In the following discussion, the convention of Chalmers et al (1971) will be adhered to. Thus ammonia means total ammonia, NH_4^+ refers to the ammonium ion and NH_3 to free ammonia. The suffix 'N' applied to any of these terms refers to the nitrogen component of that molecule or ion.

The ammonia produced by the degradation of dietary and endogenous compounds may follow one of two pathways, either being metabolised by the host animal or providing the major nitrogenous nutrient for

the synthesis of microbial protein (Smith, 1969; 1975). Research into levels of ruminal ammonia induced by feeding different diets has therefore been concerned with the potential conflict between avoiding excessive levels which would saturate the former (Chalmers et al, 1971; Davidovich, Bartley, Bechtle and Dayton, 1977) and at the same time ensuring that levels are adequate for the optimal functioning of the rumen microbial population (Mehrez, Ørskov and McDonald, 1977; Slyter, Satter and Dinius, 1979; Wallace, 1979; Mizwicki et al, 1980).

Chalmers et al (1971) investigated ammonia toxicity which had been observed when urea or ammonium salts were fed but which had not been encountered from feeding proteins, even at ruminal ammonia N concentrations >2000 mg l^{-1}. These workers confirmed previous observations that movement of ammonia across the rumen wall was passive and that the membrane was more permeable to NH_3 than to NH_4^+. The concentration of NH_3 was calculated by the Henderson - Hasselbach equation, from the pH and the ammonia concentration of rumen liquor. When intraruminal administrations of urea or casein were given to a ewe being fed a maintenance ration of hay and cereals, the peak ruminal ammonia N concentration was higher for the latter (1350 mg l^{-1}). However, the addition of 20 g of urea elevated the pH of the rumen liquor to a peak of 7.8, and 7 h after administration the pH had only fallen to 7.2. In contrast casein caused an initial drop in pH from 6.8 to 6.1 and after 2 h the pH had stabilised at its original value. These differences were reflected in the calculated NH_3N levels which, at a peak of 500 mg l^{-1}, were more than five times as high following urea, rather than casein, administration. Chalmers and her fellow workers concluded that the variable ruminal ammonia concentration:

ammonia toxicity relationships observed in the past, reflected differences in rumen liquor pH. They also demonstrated that ammonia toxicity occurred when the ammonia concentration in portal blood vessels was lower than that required to saturate the liver's ability to detoxify the ammonia by converting it to urea. This resulted from the passage of a portion of the NH_3, diffused from the rumen, via the peritoneal fluid to the jugular vein without traversing the liver.

The site of absorption of ammonia from the gastrointestinal tract appears to be contentious. Smith (1975) contended that high ruminal ammonia concentrations were not 'wasteful' since absorption from the organ was small, and that correlations between plasma urea or portal blood ammonia levels and ruminal ammonia levels were due to absorption posterior to the reticulo-rumen. Davidovich et al (1977), when investigating ammonia toxicity induced by administration of urea or gaseous NH_3, found that the greater part of ammonia absorption occurred in the rumen and, to a lesser extent, the omasum. The fact that ammonia N levels in duodenal digesta were relatively insensitive even to administration of toxic levels of urea (0.48 - 0.60 g urea kg^{-1} liveweight, cannulated heifer), indicated that intestinal absorption of ammonia was unlikely to contribute to the observed increase in the ammonia concentration in portal blood. These workers also demonstrated that the absorption of eructated NH_3 gas in the lungs was not a route by which ammonia could enter the bloodstream.

The concentration of ruminal ammonia needed to support microbial activity has been investigated by a range of methods which have yielded a wide range of estimates of threshold or optimum levels.

The results of some of these investigations are presented in Table 2.4.1. When such experiments are carried out *in vivo*, the diets tend to be of a semi-purified, or certainly concentrate-based, nature (Mehrez *et al*, 1977; Okorie *et al*, 1977; Slyter *et al*, 1979; Wallace, 1979). Variations in the ammonia concentration in the rumen or the incubation medium have been achieved by feeding or infusing urea (Satler and Slyter, 1974; Nicolic *et al*, 1975; Mehrez *et al*, 1977; Okorie *et al*, 1977; Slyter *et al*, 1979; Wallace, 1979; Mizwicki *et al*, 1980; Schaefer, 1980) or ammonium chloride (Erfle, Sauer and Mahadevan, 1977). Al-Rabbat, Baldwin and Weir (1971) found that the rate at which ammonia N was incorporated into microbial N, measured using ^{15}N as a tracer, was independent of nitrogen intake but dependent on energy intake in a dairy cow fed dried, pelleted, lucerne and barley. This demonstrated that the 'normal' diets used in the experiments supported ruminal ammonia N concentrations in excess of the requirement for maximum microbial protein synthesis.

Excessively high ruminal ammonia levels have been suggested as one of the factors which lead to the poor utilisation of the nitrogen in untreated silages (McDonald and Edwards, 1976). Morgan *et al* (1980a) observed a peak ammonia N concentration of 145 mg l^{-1} when feeding untreated grass silage (dry matter content = 181.0 g kg^{-1} total N = 16.0 g kg^{-1} DM) *ad libitum* to four Suffolk-cross wethers fitted with permanent rumen and abomasal cannulas. The animals were offered half their daily allowance at 09.00 h and half at 17.00 h. Peak ammonia N concentration was observed 4 h after feeding. When feeding the same silage, supplemented with rolled barley (300 g d^{-1}), or soyabean meal (66 g d^{-1} and 268 g d^{-1}) peak ruminal ammonia N concentrations of 175, 160 and 290 mg ammonia N l^{-1} respectively

Table 2.4.1 Estimates of ruminal ammonia N concentration required to maximise microbial growth

Ammonia N concentration (mg l^{-1})	Criteria for assessing adequacy	Reference
14.0	Ammonia saturation constants measured *in vitro* on pure cultures of a range of rumen bacteria	Schaeffer, Davis and Bryant (1980)
22.0	Maximisation of the protein N content of ruminal digesta in fistulated steers	Slyter *et al* (1979)
50.0	Maximisation of the protein N content of bovine ruminal digesta cultured *in vitro*	Satter and Slyter (1974)
58.0	Net protein synthesis in bovine rumen fluid cultured *in vitro*. ^{15}N and ^{35}S both used as indicators of microbial synthesis	Nikolic, Jovanovic and Filipovic (1975)
70.0	Maximisation of microbial protein synthesis. Measured in cannulated wethers, with ^{35}S as a marker for microbial protein	Okorie, Buttery and Lewis (1977)
194.0	Maximisation of the disappearance of dry matter from feed samples incubated in artificial fibre bags in the rumens of wethers	Mehrez *et al* (1977)
>228.0	Maximisation of the rate of disappearance of dry matter from samples of barley incubated in artificial fibre bags in the rumens of wethers	Wallace (1979)

were observed. The rate of increase in the ruminal ammonia N concentration following feeding was also greater for these supplemented diets than for the silage alone. The same workers (Morgan *et al*, 1980b) measured ruminal ammonia N concentrations in fistulated steers

offered untreated silage at 09.00 h and 17.00 h, again fed to appetite. The silage, which was from a ley containing Italian ryegrass (Lolium multiflorum), perennial ryegrass and white clover, was wetter (dry matter = 169 g kg^{-1}), had a higher N content (24.8 g kg^{-1} DM) and was consumed in greater quantities (dry matter intake = 117.1 g kg $LW^{-0.75}$ cf. 38.0 g kg $LW^{-0.75}$ for Morgan et al 1980a) than that fed to the sheep. A peak ruminal ammonia N concentration of 260 mg l^{-1} was recorded 2 h after the food was offered. In the same trial, a pelleted complete ruminant diet containing chopped straw, barley, wheatfeed, groundnut, urea and molasses (dry matter = 850 g kg^{-1}, total N = 23.2 g kg^{-1} DM), supported a peak ruminal ammonia N concentration of 300 mg l^{-1} at a dry matter intake level which was not significantly higher than that for the silage.

McDonald and Edwards (1976) cited reduction in ruminal ammonia N levels, resulting from lower non-protein N levels in the silage, as one of the possible nutritive benefits from pre-ensiling additives. Barry (1973a) observed that formaldehyde, applied to grass at rates of 150 and 300 g kg^{-1} crude protein, depressed ruminal ammonia, and volatile fatty acid levels compared with similar, untreated, material. Beever et al (1977) ensiled perennial ryegrass (cv. S24) either untreated or treated with formalin at the rate of 6.3 l t^{-1}. The mean of the peak ruminal ammonia concentrations was higher, and occurred later, for the untreated than for the treated silages (260 mg ammonia N l^{-1}, 3.5 h after feeding and 210 mg ammonia N, 1.5 h after feeding respectively) when fed to cannulated wethers.

Donaldson and Edwards (1979, 1980) observed diurnal variations in ruminal ammonia levels when silages were fed to fistulated wethers. The silages were made from ryegrass which was ensiled untreated,

or treated with either formaldehyde (94 g kg^{-1} crude protein), formic acid and formaldehyde (1.8 kg t^{-1} ensiled and 74 g kg^{-1} crude protein respectively) or sulphuric acid and formaldehyde (1.7 kg t^{-1} and 51 g kg^{-1} crude protein respectively). The silages all had high total N levels (30.5 - 35.0 g kg^{-1} dry matter) and each additive had yielded a silage with a lower non-protein N level than the control. The silages were fed at 09.00 h and 21.00 h, but access was limited to 2 h at each meal. Because of the high total N levels, and the high specific rates of dry matter intake induced by the feeding regime, ruminal ammonia N levels were, in absolute terms, high. Peak levels occurred between 2 h and 3 h after the feed was offered and were 470, 450, 350 and 320 mg ammonia N l^{-1} for the untreated, sulphuric/formaldehyde-treated, formaldehyde-treated and formic/formaldehyde-treated silages respectively.

Morgan et al (1980b) did not detect any reduction in ruminal ammonia N levels when a wilted ryegrass silage (dry matter = 359 g kg^{-1}) was fed in place of unwilted material to wethers. The total and soluble N contents of both silages were similar.

2.4.5 Efficiency of microbial protein synthesis

Conventionally, and in the sense used here, 'efficiency' means the ratio of microbial protein (or, more correctly, microbial N) produced to dietary organic matter apparently digested in the rumen (DOMR) (ARC, 1980).

The units most commonly used are 'g microbial N kg^{-1} DOMR (ARC, 1980) but standardisation is not complete. Chamberlain and Thomas (1978) used 'g microbial crude protein kg^{-1} DOMR', Okorie et al (1977) 'g microbial protein kg^{-1} DM intake', and Meggison, McMeniman and Armstrong (1979) 'g microbial N kg^{-1} OM truly digested

in the rumen'. In the last case, apparent digestion of organic matter in the rumen is corrected for the contribution of microbial organic matter to total organic matter in duodenal digesta. An alternative method of expressing the energetic efficiency of microbial protein synthesis, frequently used in _in vitro_ studies, is 'g microbial dry matter mole $^{-1}$ adenosine triphosphate produced' (Al-Rabbat _et al_, 1971; Satter and Slyter, 1974). This efficiency term is usually abbreviated to Y(ATP).

Measurement of the efficiency of microbial synthesis depends for its accuracy on measurement of the passage of organic matter and microbial N from the rumen. Both present technical problems, those of the latter (see Section 2.3) being greater than those of the former (see Section 2.2).

Meggison _et al_ (1979) demonstrated that a balance in the supply of energy and N was important for efficient synthesis of microbial protein. When a diet containing ground barley and chopped, alkali-treated, straw (1:1, w/w), together with urea, was fed to six Jersey cattle fitted with rumen and re-entrant duodenal cannulas the efficiency of microbial protein synthesis depended on the manner in which the nitrogenous supplement was given. Administering the urea twice daily resulted in an estimated efficiency of 21 g microbial N kg^{-1} DOMR. When the urea was infused continuously into the animal's feeding troughs, the corresponding value was 27.0 g microbial N kg^{-1} DOMR.

The form of dietary carbohydrate which, when fermented, provides the energy for the synthesis of microbial protein, has also been suggested as a determinant of the efficiency of the synthetic process. Mathers and Miller (1977) offered diets containing chopped lucerne

and rolled barley in the ratios 1:0, 2:1, 1:2, and 0:1 to wethers fitted with rumen and duodenal cannulas. Each food was supplemented with urea such that the intake of N was the same for all diets. The most efficient synthesis of microbial protein, which was detected by ^{35}S labelling, was seen with the 2:1 lucerne:barley diet for which the value was 33.2 g microbial N kg^{-1} DOMR. Barley alone supported an efficiency of only 22.9 g microbial N kg^{-1} DOMR. However, although the diets were isonitrogenous, those containing high proportions of barley required greater amounts of urea to maintain this state and thus important differences, other than the form of carbohydrate, existed. Supportive evidence was supplied by Siddons and Beever (1978) who found that increasing the ratio of alpha- to beta-linked glucose monomers in a ruminant's diet, by increasing the cereal to forage ratio, decreased the efficiency of synthesis of microbial N. However, the workers were concerned that interpretation of the results was complicated by the confounding effect of other dietary constituents. They therefore fed 'semi-purified' diets in which a basal ration of dried grass and ground maize was supplemented either with commercial cellulose or with maize starch. In this case, the carbohydrate supplement did not affect the efficiency of synthesis. However, the 'model' diets appeared not to produce the characteristic ruminal environments they were designed to imitate. Similar experimentation by Chamberlain and Thomas (1979) showed that as the ratio of hay to 'concentrate' (a 1:1 mix of maize and barley) changed from 1:0 through 5:2 to 3:4, the efficiency of bacterial synthesis, estimated from DAPA measurements, was unaltered. However, as the concentrate component began to dominate the diet with ratios of 1:6 and 0:1, the efficiency of this process declined.

Studies of the biochemical pathways by which ammonia is incorporated into bacterial protein have indicated that there may be important differences in energetic requirements at differing ammonia concentrations. The conventional route by which ammonia incorporation is thought to take place at moderate to high ammonia concentrations is via NADPH-dependent glutamate dehydrogenase (EC 1.4.1.4) (Erfle et al, 1977):

$$\text{alpha-ketoglutarate} + NH_4^+ + \text{NADPH} \leftrightharpoons \text{glutamate} + NADP^+$$

However at low ruminal ammonia concentrations, synthesis catalysed by an ATP-dependent enzyme, glutamine synthetase (EC 6.3.1.2), is seen as an important 'scavenging' system (Schaefer et al, 1980):

$$\text{glutamate} + NH_4^+ + \text{ATP} \leftrightharpoons \text{glutamine} + \text{ADP} + Pi$$

With silages, the fact that much of the N is present in a soluble and readily available form, whereas fermentable carbohydrate supply may be limited, led Armstrong (1973) to conclude that the energetic efficiency of microbial protein synthesis would be lower than for other diets with which the supply of substrates to the rumen microbes was more balanced. Armstrong (1980) subsequently collated measurements of microbial N production which had been determined in vivo in cattle and sheep consuming a range of foodstuffs. In cattle, the mean value for silages was 25.9 g microbial N kg^{-1} DOMR, whereas for all other diets the average was 33.7 g microbial N kg^{-1} DOMR. A similar situation was encountered in sheep, with mean values of 24.5 and 35.8 g microbial N kg^{-1} DOMR for 'silage' and 'other diets' respectively. Beever (1980), when summarising earlier work, demonstrated important differences in the supply of degraded carbohydrate and degraded N when fresh grass, wilted silage or unwilted silage were fed to sheep. The efficiencies of synthesis recorded here

were, in absolute terms, higher, but the same trend was apparent with fresh grass giving higher values than unwilted or wilted silage (53, 41 and 39 g microbial N kg^{-1} DOMR respectively). Other experiments demonstrated a clearer difference between unwilted and wilted silages in terms of efficiency of microbial synthesis with values of 167 and 113 g amino acid kg^{-1} DOMR respectively. In experiments which have been described previously (see Section 2.4.3), Thomas et al (1980) obtained estimates ranging from 10.9 to 22.7 g bacterial N kg^{-1} DOMR for ryegrass silages treated with formic acid at ensiling. Supplementing the silage which gave the highest efficiency, with barley, raised the value to 25.8 g bacterial N kg^{-1} DOMR.

3. GENERAL EXPERIMENTAL TECHNIQUES

3.1 INTRODUCTION

The work herein described consisted of a series of four metabolism experiments conducted between March 1981 and July 1983. The experiments differed with respect to the diets fed and the individual cannulated sheep employed. There were also differences in the particular measurements made, and in the management of the animals. However, the experiments were fundamentally, and in most important details, similar and these areas of commonality are considered first.

3.2 EXPERIMENTAL DESIGN AND PROCEDURE

Each experiment was conducted using six sheep (see Section 3.3) which were fed three test diets. For each experiment a cross-over design, consisting of two 3 x 3 latin squares, was adopted such that in each of the three experimental periods each diet was consumed by two sheep. The diet allocation within each period ensured that the design was balanced with respect to residual effects (Cochran and Cox, 1957). The actual design is illustrated in Fig 3.1.

Period \ Sheep	1	2	3	4	5	6
1	A	B	C	B	C	A
2	B	C	A	C	A	B
3	C	A	B	A	B	C

Figure 3.1 Typical allocation of three diets, A, B and C to six sheep over three experimental periods

Each period was of 35 days' duration. During the first 14 days the sheep were introduced to, and subsequently maintained on, their allocated diet. At this time the animals were housed, unrestrained,

in individual pens provided with clean drinking water and straw bedding. On day 15 the sheep were weighed, fitted with webbing harness for separate collection of faeces and urine and placed in wooden metabolism crates. Once in the crates the animals had free access to food and water, they could lie or stand at will, but could not turn around. Between days 15 and 21 dietary intake was recorded and ruminal digesta samples were acquired to provide information on diurnal variation in ruminal metabolism (see Section 3.5). On day 22, a total-collection digestibility trial was started, dietary intake and faecal and urinary output being measured for seven days (see Section 3.6). While this trial was being conducted, on day 25, the sheep were connected to a metered gravity flow system and continuous infusion of the dual-phase marker of digesta flow-rate began (see Section 3.7.1). The final faecal and urinary samples of the metabolism trial were collected on day 31 and, on the same day, collection of abomasal digesta commenced (see Section 3.7.2). Dietary intake and faecal output were monitored until the end of the period, the latter to provide information on the recovery of administered flow-rate marker. At the end of the period, sheep were removed from their crates, weighed and returned to their pens where they were gradually introduced to their new diets.

3.3 EXPERIMENTAL ANIMALS

All sheep used during the four experiments were castrated male animals fitted with permanent cannulas which allowed access to the rumen and abomasum. The rumen cannula was of rigid PVC construction with a circular orifice of 50 mm internal diameter which could be occluded by a screw-fit cap. The cannula providing access to the abomasum was a simple 'T-piece' fitment, again of rigid construction,

with an internal diameter of 15 mm and sealed by a push-fit rubber bung. All surgical modifications were carried out by veterinary surgeons from the Royal (Dick) School of Veterinary Studies, Edinburgh.

The animals used could all be considered to be mature. No surgery was performed on sheep less than eighteen months old and such animals were at least 20 months old before they were used experimentally. The sheep were cross-bred animals of varying parentage being, in the main, of 'Greyface' (Suffolk x Scotch half-bred) and 'Cheviot' type. Over the four experiments the weights of the sheep used ranged from 39 to 75 kg.

3.4 DIETS

A total of eight ryegrass silages and a single dried, pelleted, grass diet were used during the course of the experimental programme. Details of the preparation and storage of these diets are presented in Sections 4.2, 5.2, 6.2 and 7.2 for experiments one, two, three and four respectively. The corresponding compositional parameters are given in Sections 4.3, 5.3, 6.3 and 7.3.

3.4.1 Analyses

Dry matter

The dry matter (DM) of the silages and the dried grass were determined by drying to constant weight at 100°C in a forced draught oven. Additionally, the dry matter of the silages was determined by a toluene distillation technique (Dewar and McDonald, 1961) with a correction for the ethanol content of the distillate (Henderson, 1978)

pH	The pH of the silages was measured in a de-ionised water macerate using a combination pH electrode (Playne and McDonald, 1966)
Volatile fatty acids	The formic, acetic, propionic and butyric acid contents of the silages were determined by gas liquid chromatography (Kerr and Henderson, 1975)
Lactic acid	The lactic acid content of the silages was determined by the method of Barker and Sommerson (1941)
Ethanol	The ethanol content of the silages was determined by the steam distillation technique of Kent-Jones and Taylor (1954)
Modified acid detergent fibre	Modified acid detergent fibre was determined gravimetrically by the method of Clancy and Wilson (1966)
Water-soluble carbohydrate	Water-soluble carbohydrate levels were determined in each diet using the method of McDonald and Henderson (1964)
Organic matter	The ash content of each diet was determined by oxidising a sample in a laboratory furnace. Organic matter content was calculated as DM content less ash content
Total N	The total N content of each diet was determined by the Kjeldahl digestion/distillation technique (Horwitz, 1975)
Protein N	The N fraction, extractable in boiling water, was determined by the Kjeldahl technique

(Macpherson, 1952). Protein N was calculated as total N less this hot water-soluble N fraction

Ammonia N — The ammonia N content of the silages was determined in a cold water extract by the technique of McDonald et al (1960)

Gross energy — The gross energy (GE) content of each diet was determined by adiabatic bomb calorimetry (McDonald et al, 1973)

3.5 MEASUREMENT OF INTRA-RUMINAL METABOLISM

On days 17 and 18 of each experimental period, representative rumen liquor samples were obtained from each sheep to allow measurement of several indices of intra-ruminal metabolism. The pH and the ammonia N content of the liquor were determined immediately after sampling, whereas aliquots were frozen and retained for subsequent determination of volatile fatty acid concentrations.

3.5.1 Procedure

On days 17 and 18 of each experimental period, samples of the contents were acquired from the ventral sac of the rumen of each sheep. The timetable of the sampling regime is shown schematically in Fig 3.2. Samples were obtained under negative pressure using a hand-operated vacuum pump. The open end of the system terminated in a flexible polythene tube, of approximately 10 mm internal diameter, which was inserted through the rumen cannula into the ventral sac. Whenever possible at least 100 ml of rumen contents were removed from each sheep at each sampling time.

When all six samples for any one sampling time had been obtained, their pH values were determined immediately. The samples were then filtered through four layers of muslin to remove large

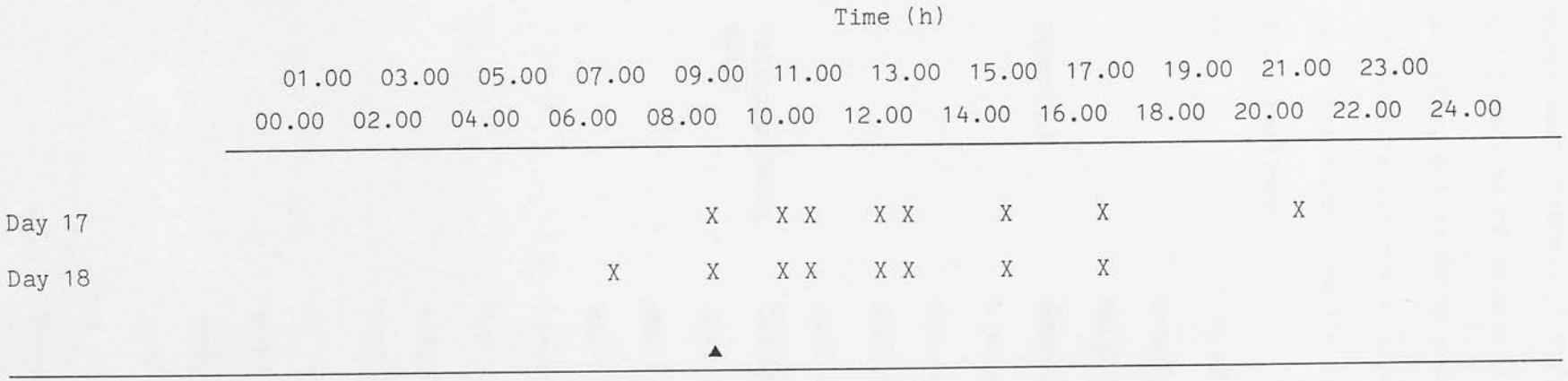

Figure 3.2 Schematic timetable of the rumen sampling regime
Rumen sample taken = X ▲ =
Feed residue removed, fresh 24 h allocation provided

particulate material. Fifty ml sub-samples of this filtrate were added to 1 ml of saturated mercuric chloride, to arrest microbial activity, before being stored at -18°C for subsequent determination of volatile fatty acid concentrations. A 5 ml sample of the remaining filtrate was taken for the determination of ammonia N content.

3.5.2 Analyses

pH — The pH of the unfiltered rumen contents was determined using a Corning-EEL Model 10 pH meter fitted with a Russell combination electrode

Ammonia N — Ammonia N concentration was determined in a 5 ml sample of filtered rumen contents using the microdiffusion technique of Conway and O'Malley as described by Chalmers , Cuthbertson and Synge (1954).

Volatile fatty acids — Determination of individual volatile fatty acid concentrations, in strained rumen liquor, was routinely performed at the end of each experiment. Samples had thus been stored for between three and 13 weeks before being analysed. For analysis, samples of strained rumen liquor were constructed by combining two 5 ml aliquots from the same sheep taken at the same time on consecutive sampling days. For the 07.00 h and 21.00 h sampling times where only a single sample was available from each sheep (see Fig 3.2), this sample alone was used as the source of a 10 ml aliquot for analysis.

Protein was precipitated from the liquor by

mixing with 2 ml metaphosphoric acid (M in 10 M sulphuric acid) and refrigerating at 0 - 4°C for 30 min. The resultant proteinaceous precipitate was separated by centrifugation and the clarified supernatant was retained for subsequent determination of its acetic, propionic, iso-butyric, n-butyric, iso-valeric, n-valeric and hexanoic acid contents by gas-liquid chromatography. Details of this analysis are shown in Table 3.1 below

TABLE 3.1 **Operating parameters of the gas-liquid chromatograph used to determine individual volatile fatty acid concentrations in deproteinised strained rumen liquor**

Gas-liquid Chromatograph		Pye-Unicam Series 304
Injector temperature		145°C
Column	Length	2.43 m
	Packing	SP1200
	Temperature	125°C
Detector	Type	Flame ionisation
	Temperature	150°C
Gas pressures	Carrier (Ar)	11 psi
	Fuel (H)	21 psi
	(Air)	11 psi

3.6 MEASUREMENT OF DIETARY DIGESTIBILITY

In each experiment the in vivo digestibility of the diets being fed was assessed directly, by reference to the amount of food consumed and the concomitant production of faeces, over a period of seven days. A conventional lag of three days between the first measured

offering of feed and the start of the faecal collection was adopted and this phase of the experimentation consequently occupied 10 days, days 22 - 31, of each experimental period.

3.6.1 Procedure

The appropriate daily allowance of feed, of known dry weight, was offered to each animal at 09.00 h, immediately after the residue from the previous day's allowance had been removed.

The sheep were harnessed for the separate collection of faeces and urine. Faeces voided over a 24 h period were removed at approximately 09.30 h and stored, undried, at -18°C. At the end of the seven day collection period the bulked sample from each animal was thawed, weighed, thoroughly mixed and sub-sampled to provide representative fractions for subsequent determination of dry matter, organic matter, total N and gross energy contents. Urine was collected continuously in 25 l capacity polythene bottles positioned under the sheep's metabolism crates. A large rubber funnel, attached to the webbing harness and held in close contact with the animal's underside guided all excreted urine, via rubber tubing, to the collection vessel. The urine was maintained at a pH of 2 - 3, by the addition of sulphuric acid, to avoid loss of ammonia N. At the end of the seven day collection period, which corresponded with that for faecal collection, the total weight of urine produced was measured and the material was sampled to provide representative aliquots for subsequent determination of its gross energy and total N contents.

3.6.2 Analyses

Dry matter — The samples of feed residues and fresh, thawed, faecal samples were routinely dried for 48 h at 100°C in a forced draught oven. For small residues of dried grass or wilted silage, a 24 h

drying period was considered sufficient

Total N — The total N contents of the fresh faeces and the acidified urine samples were determined in the Central Analytical Laboratory of the Edinburgh School of Agriculture using the technique of Crooke and Simpson (1971)

Organic matter — Faecal organic matter was determined in the School's Central Analytical Laboratory by oxidising the sample in a furnace.

Gross energy — The gross energy of faecal and urinary samples was determined by adiabatic bomb calorimetry (McDonald et al, 1973)

By combining information obtained during this digestibility measurement phase with previously determined dietary parameters, the apparent digestibility of the dietary dry matter, total N and gross energy could be calculated. Urinary N excretion could also be assessed in terms of its relation to dietary intake.

3.7 MEASUREMENT OF DIGESTA FLOW

The rate of flow of digesta to the main absorptive regions of the digestive tract was assessed by reference to the dilution, within the digesta, of a dual-phase marker which was infused continuously (Faichney, 1975b). A solution of tris (1, 10-phenanthroline) ruthenium (II) chloride (Ru-P) was used as the solid phase marker and this was combined with a solution of the chromium complex of ethylenediamine tetra-acetic acid (Cr-EDTA) to constitute the dual-phase system.

3.7.1 Procedure

The stock Ru-P was prepared by the method of Macrae and Evans

(1974), whereas the analagous Cr-EDTA was prepared according to the procedure of Binnerts et al (1968). The Ru-P stock solution contained approximately 2000 mg Ru kg^{-1} and was diluted 1:19 (v/v) with deionised water to provide a working solution containing 100 mg Ru kg^{-1}. Similarly, the Cr-EDTA stock solution ($\simeq$ 2770 mg Cr l^{-1}) was diluted 1:1.25 (v/v) to produce a working solution with a chromium concentration of 1231 mg l^{-1}. When required, these working solutions were combined in equal volumes to provide a mixture for infusion with nominal ruthenium and chromium concentrations of 50 and 615 mg l^{-1} respectively.

The Ru-P/Cr-EDTA mixture was administered into the sheep's rumen via the permanent cannula. Individual polythene reservoirs, of 1.5 l maximum capacity, were used for each animal. These reservoirs were placed on a wall-mounted shelf, positioned behind the metabolism crates, 2.5 m above floor level and approximately 1 m above the height of the rumen cannulas. The marker solution was conducted from the reservoir to the rumen along clear vinyl tubing of 6 mm internal diamter. The tubing passed through a central hole drilled in the screw fitting cannula cap and was, at that point, reinforced against abrasion by a sleeve of rubber tubing. The distal end of this transmission tube terminated within the rumen, projecting between 0.15 and 0.20 m into the organ. The flow of marker into the animal's goastrointestinal tract was metered by placing a simple, multi-channel, fixed speed, axial peristaltic pump (Delta-C, Watson-Marlowe Ltd) in-line between the reservoir and the transmission line. Using 3 mm internal diameter silicon rubber tube pumping elements, appropriately tensioned, this system provided sufficiently accurate (mean channel outputs ranged from 339.0 to 550.6 g 24 h^{-1} over the series

of experiments) and precise (the SEM for any given channel over any one infusion phase was typically in the range 5 - 10 g 24 h^{-1}) infusion of the marker.

When full, the reservoirs provided sufficient infusate for between two and three days' continuous infusion. Individual weights before and after replenishment were recorded so that the infusate for each sheep could be calculated. A representative sample of the liquid in the reservoirs was also taken at this time and these samples were retained for subsequent measurement of their ruthenium and chromium contents.

The flow-rate marker infusion phase commenced on day 25 of each experimental period, when the reservoirs were filled and the pump tubing was primed. To hasten the attainment of stable marker concentrations in the digesta, a priming dose of marker solution, corresponding to approximately one half of the envisaged 24 h infusate, was administered by syringe immediately before continuous infusion commenced (Faichney, 1975b; B. Mayes, HFRO, personal communication). Infusion was stopped at 08.30 h on day 34 of each period, by which time all collections of digesta and faeces had been completed.

3.7.2 Sampling

Samples of abomasal digesta were taken from the sheep on days 31, 32, 33 and 34 of each experimental period. The sampling regime adopted was designed to ensure that the final composite sample from each animal was wholly representative of the material flowing through the alimentary tract in a 24 h period, and that the sampling burden was spread to avoid any major physiological dysfunction in the sheep. The first criterion was fulfilled by dividing the 24 h day into eight 3 h periods and sampling digesta at the mid-points of these periods.

Digesta samples were therefore taken from each sheep at 01.30 h, 04.30 h, 07.30 h, 10.30 h, 16.30 h, 19.30 h and 22.30 h. A maximum of 125 ml of digesta was removed at each sampling time and, to fulfil the second criterion, the acquisition of digesta was spread over a period of 63 h. The first sample was taken at 10.30 h on day 31 and sampling then proceeded at 9 h intervals ending at 01.30 h on day 34 when the eighth sample was acquired from each animal. The abomasal digesta sampling schedule is illustrated in Fig. 3.3.

The sampled liquid digesta (SLD) was collected in bottles of 1.5 l capacity and was maintained at 4°C during the collection phase. Processing of the digesta, which is illustrated in Fig. 3.4, commenced immediately after the final samples for the period had been taken. The bulked SLD collection from each animal was divided, after thorough mixing, into two aliquots. One aliquot from each sheep was then centrifuged (MSE 4L, 4 x 1 l swing-out head, 2500 rpm/30 min/4°C) and the supernatant discarded. The residual pellet of centrifuged liquid digesta (CLD) was washed from the centrifuge bottle with a measured volume of distilled water. The resultant slurry was termed dilute centrifuged liquid digesta (DCLD). DCLD and SLD samples were retained for subsequent determination of their total N, ammonia N dry matter and organic matter contents. The remaining DCLD and SLD materials were freeze-dried and then ground in a laboratory hammer mill fitted with a 2 mm screen. This dried, ground, sampled (SD) and centrifuged (CD) digesta was used as a source of sample material for subsequent measurement of ruthenium, chromium and nucleic acid contents (see Section 3.7.3 below).

Total faecal collections were made during the final week of each period. Collections were of 48 h duration, encompassing days

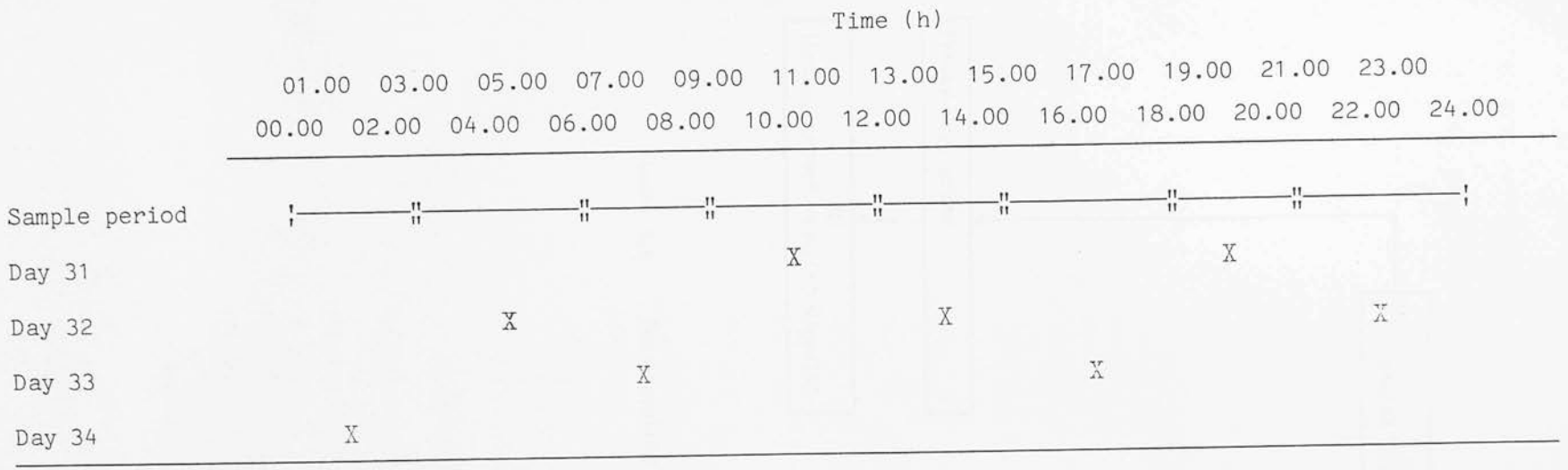

Figure 3.3 Schematic representation of the abomasal digesta sampling regime adopted for the final week of each period in Experiment One (week four) and Experiments Two, Three and Four (week five).
Abomasal digesta sample taken = X

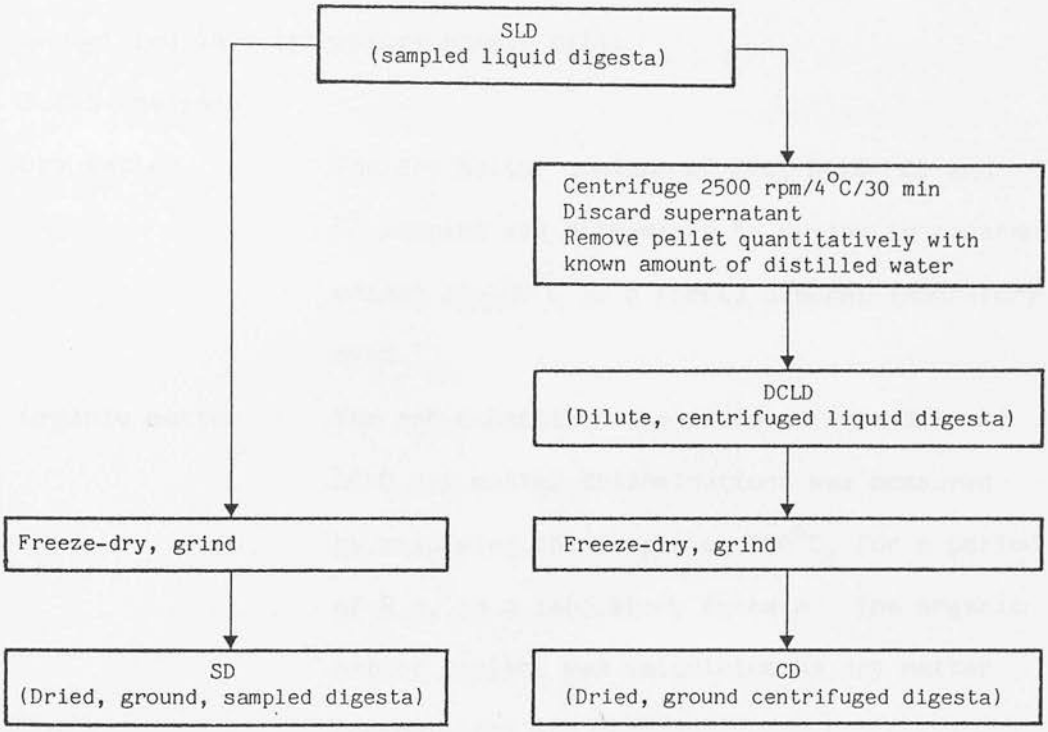

Figure 3.4 Post-sampling processing of abomasal digesta

33 and 34. The faeces were stored at -18°C until collection was complete, when the weight of fresh material produced was recorded. The faecal samples were then dried at 100°C in a forced draught oven and milled in a laboratory hammer mill.

3.7.3 Analyses

Dry matter — The dry matter content of SLD, DCLD, SD and CD samples was determined by drying to constant weight at 100°C in a forced draught laboratory oven

Organic matter — The ash content of the residues from SLD and DCLD dry matter determinations was measured by oxidising the sample at 550°C, for a period of 8 h, in a laboratory furnace. The organic matter content was calculated as dry matter content less ash content

Total N — Total N content was determined in SLD and DCLD samples of approximately 1 g dry weight using the Kjeldahl digestion-distillation technique (Horwitz, 1975)

Ammonia N — Digesta ammonia N content was measured in diluted samples of SLD and DCLD using a selective ion electrode (EIL ammonia probe, Model 8002). The non-ammonia N content of digesta was calculated as total N less ammonia N content

Flow-rate markers — The ruthenium and chromium contents of the infused marker solution, the SD and CD digesta samples, and the dried, milled faeces were determined by the Analytical Chemistry Department of the

Hill Farming Research Organisation. Ruthenium content was determined by x-ray fluorescent spectrophotometry (Macrae and Evans, 1974; Evans et al, 1977). Chromium content was determined using atomic absorption spectrophotometry

Nucleic acid

The nucleic acid content of freeze-dried abomasal digesta samples (SD and CD) was determined by a hot sodium chloride extraction procedure based on those of Guinn (1966) and Ling and Buttery (1976 and J R Ling, personal communication). Whereas these techniques are similar in principle there are important differences which may materially affect the values determined, and the technique as employed in the present studies is described in detail.

The procedure adopted is shown schematically in Fig. 3.5. The range of sample weight used was 99 - 365 mg DM (step A). Within this range the ethanol and ehtanol:NaCl purifying solution volumes appeared to be adequate. This tolerance was also noted by Guinn (1966). The separation of precipitated, extracted, nucleic acids (step G) could be effected by this relatively mild centrifugation regime when ethanol was used as the precipitant. Both Guinn (1966) and Ling and Buttery (J R Ling, personal communication) used considerably higher centrifugal force, but employed cold trichloracetic acid as the precipitant.

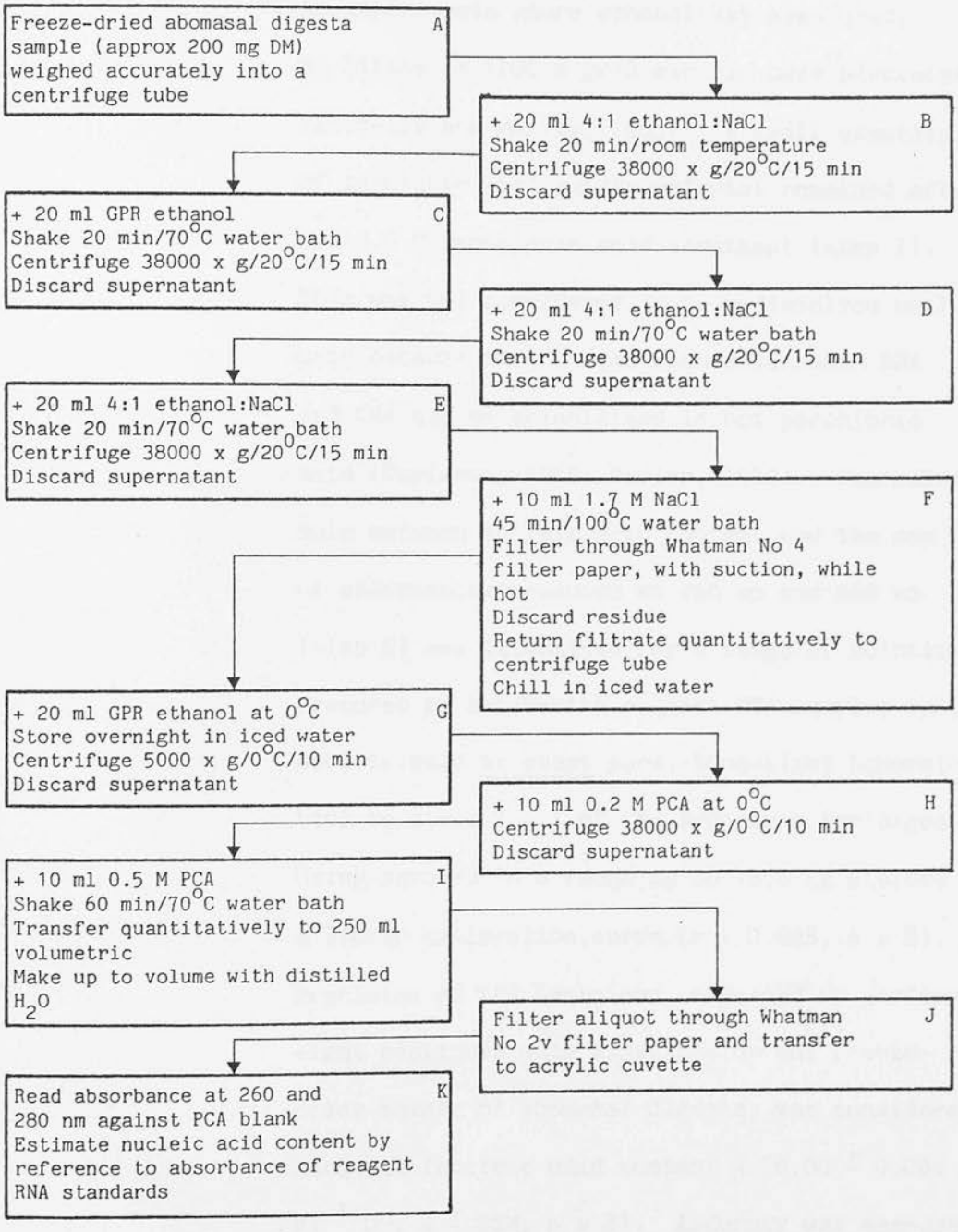

Figure 3.5 Schematic representation of the purification and extraction method used to determine nucleic acid content of freeze-dried abomasal digesta samples

In experiments where ethanol has been used, as little as 1200 x g/10 min has been advocated (Zscheile and Murray, 1963). A small quantity of insoluble particulate material remained after hot 0.5 M perchloric acid treatment (step I). This was not considered to be undissolved nucleic acid because of the ease with which both RNA and DNA may be solubilised in hot perchloric acid (Davidson, 1969; Parish, 1972). The relationship between nucleic acid content and the sum of absorbances measured at 260 nm and 280 nm (step K) was determined for a range of solutions prepared by subjecting reagent RNA samples (ribonucleic acid ex yeast pure, Koch-Light Laboratories Ltd) to steps F - K of the procedure for digesta. Using samples in a range up to 16.0 mg yielded a linear calibration curve ($r = 0.998$, $n = 8$). Precision of the technique, assessed by performing eight replicate determinations on one freeze-dried sample of abomasal digesta, was considered adequate (nucleic acid content = 18.00 ± 0.264 g kg^{-1} DM; $x \pm$ SEM, $n = 8$). Accuracy was assessed by calculating the recovery of reagent RNA added to similar digesta material. The value obtained ($87.0 \pm 3.50\%$; $x \pm$ SEM, $n = 8$) was used to correct subsequent determinations of nucleic acid in digesta.

The microbial N content of the freeze-dried

SD and CD samples was derived factorially from the measured nucleic acid value. Firstly, observed nucleic acid content was multiplied by a factor of 1.15 to compensate for incomplete recovery. This corrected nucleic acid content was then expressed as a nucleic acid N content, using a value of 141.4 g N kg^{-1} for the proportion of N in mixed microbial nucleic acid (Ellis and Pfander, 1965; McAllan and Smith, 1969). The microbial N content was then calculated by assigning a value of 0.157 to the ratio of microbial nucleic acid N to total microbial N (Elllis and Pfander, 1965; Smith et al, 1968; Ling and Buttery, 1978; Schelling, Koenig and Jackson, 1982; Zinn and Owens, 1982; see Review of Literature and Discussion).

This factorial derivation of microbial N is shown below:

$$\text{microbial N (g kg}^{-1}\text{ DM)} = \frac{\text{nucleic acid (g kg}^{-1}\text{ DM)} \times 1.15 \times 0.1414}{0.157}$$

3.7.4 Calculations

The principles underlying the use of a dual phase marker system to estimate the concentrations of constituents of true digesta (TD) flowing past the point of sampling, within the lumen of the digestive tract, are described in Appendix A. Here, the process is presented in a simplified form.

i) The flow-rate marker concentrations in SD and CD, in combination with the flow-rate marker infusates*, allowed the computation

* No correction was applied to observed infusates to account for incomplete recovery of the markers in the faeces.

of a reconstitution factor. This describes the extent to which SD must be supplemented by, or deprived of, CD to reconstitute TD. The flow-rate marker concentration in TD was then evaluated.

ii) Marker infusate (gd^{-1}) was divided by TD marker concentration ($g\ kg^{-1}$) to yield TD flow rate ($kg\ d^{-1}$).

iii) Daily rates of flow of digesta constituents were then calculated by firstly estimating their concentrations in TD, as in step i) and then multiplying the resultant concentrations by the flow rate evaluated in step ii).

In addition to the rates of flow of digesta constituents, the degradability of dietary N was estimated from the measured total N intake, and total N and microbial N flow rates (ARC, 1980).

3.8 STATISTICAL TECHNIQUES

The data generated by the animal experimentation were examined using analysis of variance techniques described by Cochran and Cox (1957). The necessary calculations were performed with the aid of a computer programme, written in BASIC and run on a Series 3000 Commodore Pet, or a Comart Communicator, microcomputer.

Provided with the observed values which, for any one parameter in an experiment, numbered, at most, 18, and a number identifying the diet 'responsible' for each value, the following information was generated:

i) The data as analysed (including estimates of any missing values - see Cochran and Cox, 1957).

ii) An analysis of variance table indicating the level of significance of sheep, period and diet effects.

iii) The arithmetic mean values for each diet together with the standard errors of these means and of the difference between any pair.

iv) The magnitudes of the differences between all combinations of the diet means.

v) The critical values to be exceeded by the inter-mean differences to achieve significance at three levels of probability.

4. EXPERIMENT ONE

4.1 INTRODUCTION

Although popular, the use of pre-ensiling additives is not universal in the United Kingdom (Minister, 1978). Much of the grass which is ensiled without additive treatment is prewilted (MAFF, 1977; ADAS, 1985), but the water-soluble carbohydrate content of ryegrass at more advanced stages of growth is high enough to allow unwilted, untreated silage to be successfully made (Henderson and McDonald, 1976). In the first experiment of the current series, the digestion and metabolism of silage in this, its simplest, form was studied.

When assessed on the basis of digestible nutrient content, a ruminant diet with a higher content of total N is likely to be considered of greater value than a similar diet with a lower content. To assess the effects of such a difference on more critical measures of the nutritive value of a diet, this experiment was conducted using two silages differing widely in their content of total N. The difference was produced by varying the amounts of nitrogenous fertiliser which were applied to the swards from which the silages were made.

4.2 METHODS AND MATERIALS

4.2.1 Experimental design and procedure

Experiment One was conducted from 23 March to 14 June 1981. Although the design and procedure were similar, in principle, to those outlined in Section 3.2, 3.3, 3.4, 3.6, 3.7 and 3.8, this first experiment differed in several respects from those that followed.

The seven sheep used in the experiment were numbers 23, 88, 91, 135, 193, 440 and 1280. The average weight of these animals over the course of the experiment was 54.1 kg, with minima and maxima of 39.5 kg and 66.45 kg. The allocation of diets for each animal is shown in Figure 4.1.

			Column			
	I	II	III	IV	V	VI
Sheep	440	1280	91	88	23/193	135
Period						
1	DG	A	B	DG	A	B
2	A	B	DG	B	DG	A
3	B	DG	A	A	B	DG

Figure 4.1 Allocation of three diets, dried grass (DG), silage with low content of total N (A) and silage with high content of total N (B), to six sheep over three experimental periods in Experiment One. No measurements were obtained from sheep 193 in period 1

The sheep were kept in wooden metabolism cages for the duration of the experiment. Each period was of 28 days' duration, with the animals being introduced to, and subsequently fed, their allocated diet during the first 14 d. On day 15, the sheep were weighed and the total-collection digestibility trial was started. This trial followed the schedule described in Section 3.6 above and differed only in that it occupied days 15 - 24 of a 28-day period rather than days 22 - 31 of a 35-day period. On day 19, the sheep were connected to the gravity-fed system and continuous, metered,

infusion of the flow rate marker solution was started. With the exception that this infusion commenced on day 19 and was stopped at 08.30 h on day 27, the procedure described in Section 3.7.1 above was adhered to. Samples of abomasal digesta were taken from the sheep on days 24, 25, 26 and 27, according to the timetable shown in Figure 3.3 for days 31, 32, 33 and 34 of the longer periods in the later experiments. Processing of the samples of abomasal digesta followed the procedures described in Section 3.7.2 above. The collection of faeces, to allow estimation of the recovery of administered Ru and Cr, was carried out over a period of 72 h encompassing days 25, 26 and 27 of each experimental period.

The data from the animal measurements were examined using analysis of variance techniques described in Section 3.8 above.

No measurements of ruminal metabolism (Section 3.5) were made in Experiment One.

The experimental design shown in Fig 4.1 is balanced with respect to residual effects (albeit incompletely, since a diet is never preceded, or followed, by itself) and allows variation in observed values to be apportioned to the effects of diet, period and sheep. For a variety of reasons, not all parameters were recorded for each sheep x period combination, and the six animals which started the trial did not remain in the experiment until the end of Period Three. Statistical techniques can be used to deal with simple missing values (Cochran and Cox, 1957). For animals which have to be removed from the trial the alternatives are either to continue the experiment with the depleted group or to replace the ailing sheep with similar animals which have been held in reserve. If the first option is used and the original design retained, the

number of missing values which must be estimated in the analysis of variance means that the likelihood of identifying significant effects attributable to the imposed treatments, is reduced. With the second option, the maximum amount of data are collected, but the usefulness of the mean square term associated with sheep is reduced.

In Experiment One, the following disruptions to the planned routine occurred:

(i) The intake of dry matter by Sheep 91, during the digestibility trial phase of Period One, was exceptionally low (256 g d^{-1}) and the resulting coefficients of digestibility were considered so unrepresentative that they were treated as missing values in the statistical analyses.

(ii) Sheep 23 refused almost all the silage offered to it during Period One (recorded intake was 27 g DM d^{-1} during the digestibility trial phase). The animal lost weight and condition, and was taken off the trial before the end of the period. No measurements were therefore obtained for Silage A in Column V. Sheep 23 was replaced by Sheep 193 for Periods Two and Three.

(iii) The concentrations of Ru and Cr, measured in the digesta samples taken from Sheep 91 in Period Three, were unrealistically low. (For possible explanations see Section 8). The rates of flow calculated from these marker concentrations were, in turn, far higher than those from any other sheep x diet

combination. All rates of flow, and parameters derived from them, were therefore regarded as being missing values.

4.2.2 Diets

Three diets were offered in Experiment One, two grass silages and the pelleted, dried grass which was fed in each experiment.

4.2.2.1 Silages

The herbage for both silages was harvested from a ley of Italian and perennial ryegrass on 11 June 1980. Half of the harvested area had received an application of pig and poultry slurry, at the rate of 49 t ha^{-1}, on 24 April 1980. This slurry had a dry matter content of 46.4 g kg^{-1} and N, P and K contents of 35.4, 21.2 and 49.7 g kg^{-1} DM respectively. It thus supplied 81 kg N ha^{-1}. This was in addition to N applied as ammonium nitrate to the whole area. The slurry application was designed to produce herbage with a higher content of total N than was present in the untreated grass.

The crop was cut with a drum mower and lifted immediately using a precision-chop forage harvester. The herbage was ensiled in concrete bunker silos 1.6 m wide, 2.8 m long and 1.6 m deep. One silo was filled with about 4 t of material harvested from the area which had received no slurry and a second was filled with 4.5 t of grass from the area which had been manured. As the silos were being filled the herbage was consolidated manually, and when filling was complete, the ensiled material was covered with polythene sheeting which was sealed and weighted with sandbags.

The silos were opened on 19 February 1981 after an ensiling period of 253 d. Surface spoilage was removed and approximately

1 t of silage was taken from each silo and placed on a clean concrete floor. Each silage was mixed and representative samples were taken for subsequent analysis (see Section 3.4.1). Daily rations of silage for individual sheep (about 1 kg DM) were weighed into polythene bags which were then sealed. These bags contained 4.5 kg of Silage A made from the untreated grass, or 5.5 kg of Silage B made from the grass to which the slurry had been applied. These daily rations, 170 for each silage, were stored at -20°C until required. Silage was thus in refrigerated storage for between 30 and 110 days before being thawed and fed to the sheep.

4.2.2.2 Dried grass

Pelleted dried grass was fed throughout the series of experiments as a control diet. It was chosen because a sufficient bulk, of uniform composition, could be obtained at the start of the experiments. The grass pellets were stored in a pest-proof container, and at intervals during the course of the experiment were placed in polythene bags containing the desired daily ration of 1 kg dry matter (1.14 kg fresh matter).

4.3 RESULTS

4.3.1 Composition of diets

The composition of the grasses from which the silages were made, during the period between the application of the slurry on 24 April 1980 and cutting the crop on 11 June 1980, are presented in Table 4.1. The compositions of the resulting silages are shown in Table 4.2.

The most striking difference in composition between the two silages was their content of total N, that for Silage A being more than one and a half times that of the other. The difference in

dry matter contents was also appreciable.

The composition of the pelleted, dried grass is shown in Table 4.3.

Table 4.1 Composition of untreated (U) and treated (S) grass in the period between the application of slurry and harvesting of the crop

	15 May		29 May		11 June	
	U	S	U	S	U	S
Dry matter (g kg^{-1})	245	207	199	173	245	192
Water soluble carbohydrate (g kg^{-1} DM)	289	175	247	225	230	167
Total N (g kg^{-1} DM)	29.3	43.3	24.0	32.0	19.1	30.4
Protein N (g kg^{-1} total N)	903	869	891	855	865	840

Table 4.2 Composition of silages

Composition ($g\ kg^{-1}$ DM unless otherwise stated)	Silage A	Silage B
Dry matter[1] ($g\ kg^{-1}$)	204.2	162.1
Dry matter[2] ($g\ kg^{-1}$)	240.0	190.7
pH (units)	3.74	3.98
Organic matter	938	930
Water-soluble carbohydrate	41	0
Ethanol	23	12
Lactic acid	113	122
Acetic acid	28.0	37.0
Propionic acid	0.3	0.4
N-butyric acid	0.2	0.1
Formic acid	2.0	0.8
Total N	18.8	29.6
Protein N	6.3	9.7
Protein N ($g\ kg^{-1}$ total N)	335	325
Ammonia N	1.5	3.4
Ammonia N ($g\ kg^{-1}$ total N)	80	115
Non-ammonia N	17.3	26.2
Non-ammonia N ($g\ kg^{-1}$ N)	920	885
Gross energy ($MJ\ kg^{-1}$ DM)	19.6	20.1

1 Dry matter determined by drying to constant weight at 100°C

2 Dry matter determined by distillation with toluene (see Section 3.4)

Table 4.3 Composition of pelleted, dried grass as offered to sheep

Composition (g kg^{-1} DM unless otherwise stated)	Pelleted, dried grass
Dry matter	882.2
Organic matter	933
Water-soluble carbohydrate	190
Total N	28.5
Protein N	23.4
Protein N (g kg^{-1} total N)	821
Ammonia N	0.3
Ammonia N (g kg^{-1} total N)	11
Non-ammonia N	28.2
Non-ammonia N (g kg^{-1} total N)	989
Gross energy (MJ kg^{-1} DM)	18.8

4.3.2 Dietary digestibility

The results of the digestibility measurements made during the third quarter of each experimental period are presented in Tables 4.4 - 4.7.

The similar compositions of the silages were reflected by the fact that no distinction could be made between the digestibilities of their dry matter, energy or organic matter. In the case of total N, however, the extent of the nutrient's digestion was significantly ($P < 0.01$) higher for Silage B. The higher digestibility combined with a higher concentration of total N resulted in a digestible crude protein content, for Silage B, 78% greater than that for Silage A. Although variability in recorded parameters of digestion was observed between sheep and between periods, none of these effects attained statistical significance ($P > 0.05$).

Note:

A common format has been adopted for the tables of results of the animal experimentation in all four experiments. For each parameter, the mean values for the diets are followed by the Least Significant Difference (LSD). This is the value which must be exceeded by the magnitude of the difference between any pair of treatment means for that difference to attain statistical significance ($P < 0.05$). In addition, the level of significance of the important comparison between the two silages, is indicated for each parameter with the following symbols: NS ($P > 0.05$), * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$).

Table 4.4 Amounts of dry matter consumed during digestibility trial

	Dry matter intake	
	$g\ d^{-1}$	$g\ kg^{-0.75}\ LW\ d^{-1}$
Silage A	1025	50.9
Silage B	863	43.1
Dried grass	1001	50.4
LSD	271.9	17.36
Significance	NS	NS

Table 4.5 Digestibility coefficients

	Dry matter	Gross energy	Organic matter	Total N
Silage A	0.737	0.745	0.763	0.681
Silage B	0.717	0.724	0.741	0.771
Dried grass	0.643	0.642	0.674	0.704
LSD	0.0430	0.0426	0.0435	0.0469
Significance	NS	NS	NS	**

Table 4.6 Concentration of digestible and metabolisable nutrients in the diets

	DOMD ($g\ kg^{-1}$ DM)	DE ($MJ\ kg^{-1}$ DM)	ME ($MJ\ kg^{-1}$ DM)	DCP ($g\ kg^{-1}$ DM)
Silage A	716	14.6	12.2	80
Silage B	689	14.5	11.8	143
Dried grass	630	12.1	9.7	125
LSD	41.5	0.86	0.71	6.9
Significance	NS	NS	NS	***

Table 4.7 Retention of nitrogen and the excretion of nitrogen and energy in the urine

	N retention ($g\ d^{-1}$)	Urinary excretion (% of intake)	
		N	Gross energy
Silage A	4.39	46.1	4.2
Silage B	4.30	62.3	5.5
Dried grass	7.28	44.9	4.9
LSD	1.758	9.16	0.79
Significance	NS	**	**

4.3.3 Rate of flow of digesta

Mean daily intakes of dry matter, recorded during the period when measurements of flow rate were being made, are shown in Table 4.8.

Intakes of dry matter during the flow rate phases of the trial were virtually identical to those in the preceding phases during which digestibility was measured (compare Table 4.4).

Apparent recoveries of the Ru and Cr in the administered marker are presented, together with the reconstitution factors, in Table 4.9.

The measured, or derived, estimates of the rate of flow of a range of nutrients in abomasal digesta are presented in Table 4.10, and in Table 4.11 the rates of flow of nutrients at the abomasum are expressed in terms of their intakes.

In Table 4.12, three related terms which express the extent to which dietary N has been incorporated into microbial N are presented. The second, corrected, degradability term uses a value of 1.5 g d^{-1} as an estimate of the flow of endogenous N at the abomasum (Smith et al, 1976).

The concentration of DOMR in the diets, and the relative importance of the ruminally digested portion of the DOM, are shown in Table 4.13. The apparent efficiency of synthesis of microbial N, expressed in two ways, is presented in the same table. In Table 4.14, the contents of non-ammonia N and degradable N in the diets are expressed per unit digestible, or ruminally digestible, organic matter.

The supply of non-ammonia N to the abomasum is illustrated in a variety of ways in Figures 4.2, 4.3, 4.4 and 4.5 In

Fig 4.2, the simplest relationship is shown, with the flow of the nutrient at the abomasum related to corresponding intake in the food. Fig 4.3 attempts to relate the specific flow of non-ammonia N (g kg^{-1} non-ammonia N intake) to the concentration of the nutrient in the DOM whereas in Fig 4.4, the same dependent variable is plotted against the content of non-ammonia N in the DOMR. Finally, in Fig 4.5, the supply of non-ammonia N is related to the concentration of ruminally degraded N in the DOMR. This last term describes the balance between the supply of N and energy for the rumen microbes. Thus, as the value for the expression "g degradable N kg^{-1} DOMR" increases, more degradable N is available per unit organic matter degraded in the rumen, and hence per unit energy available for microbial synthesis.

Table 4.8 Amounts of dry matter consumed during measurement of flow rate

	Dry matter intake	
	g d^{-1}	g $kg^{-0.75}$ LW d^{-1}
Low N silage	949	50.9
High N silage	871	43.9
Dried grass	1001	50.4
LSD	271.8	15.18
Significance	NS	NS

Table 4.9 Reconstitution factors and apparent recovery of administered markers

	Reconstitution factor 'R'	Apparent recovery (%)	
		Ru	Cr
Silage A	- 0.126	80.2	85.7
Silage B	- 0.101	79.5	86.9
Dried grass	- 0.233	82.4	91.6
LSD	0.2026	10.42	6.87
Significance	NS	NS	NS

Table 4.10 Flow of nutrients at the abomasum

	Nutrient flow (g d^{-1})				
	Dry matter	Organic matter	Total N	Non-ammonia N	Microbial N
Silage A	651	481	23.2	21.8	13.3
Silage B	497	393	20.1	18.3	6.8
Dried grass	696	557	30.7	28.5	9.5
LSD	195.9	186.2	7.16	6.92	4.76
Significance	NS	NS	NS	NS	*

Table 4.11 Flow of nutrients at the abomasum, related to nutrient intake

	Nutrient flow					
	Organic matter	Total N	Non-ammonia N			Microbial N
	(g kg^{-1} OM intake)	(g kg^{-1} TN intake)	(g kg^{-1} TN intake)	(g kg^{-1} NAN intake)	(g MJ^{-1} ME intake)	(g kg^{-1} TN intake)
Silage A	532	1283	1200	1305	1.71	683
Silage B	495	808	733	828	1.55	277
Dried grass	596	1075	999	1010	2.93	332
LSD	119.6	168.0	160.5	169.6	0.676	153.9
Significance	NS	***	***	***	NS	***

Table 4.12 Microbial N content of abomasal digesta and apparent degradability of dietary N in the rumen. The degradability terms are obtained using the equation in ARC (1980)*

	Microbial N content of abomasal digesta ($g\ kg^{-1}$ total N)	Uncorrected degradability of dietary N	Corrected degradability of dietary N
Silage A	546	0.402	0.490
Silage B	342	0.468	0.543
Dried grass	308	0.258	0.310
LSD	114.1	0.1434	0.1310
Significance	**	NS	NS

* Uncorrected degradability $= 1 - \frac{(\text{duodenal N} - \text{microbial N})}{\text{intake N}}$

Corrected degradability $= 1 - \frac{(\text{duodenal N} - (\text{microbial} + \text{endogenous N}))}{\text{intake N}}$

Table 4.13 Dietary content of DOMR and the efficiency of microbial protein synthesis

	Proportion of DOM apparently digested in the rumen	Dietary Content of DOMR ($g\ kg^{-1}$ DM)	Efficiency of synthesis of of microbial N	
			($g\ N\ kg^{-1}$ DOMR)	($g\ N\ MJ^{-1}$ ME intake)
Silage A	0.55	393	34.4	1.09
Silage B	0.61	432	17.9	0.64
Dried grass	0.60	377	26.3	0.98
LSD	0.214	153.3	16.18	0.342
Significance	NS	NS	*	*

Table 4.14 Concentration of non-ammonia N in the DOM and DOMR, and degradable N in the DOMR, of the diets

	Non-ammonia N content of diet		Degradable N content of diet
	(g kg^{-1} DOM)	(g kg^{-1} DOMR)	(g kg^{-1} DOMR)
Silage A	24.1	38.8	21.9
Silage B	36.8	51.3	31.8
Dried grass	44.8	76.3	23.8
LSD	1.56	23.52	12.83
Significance	***	NS	NS

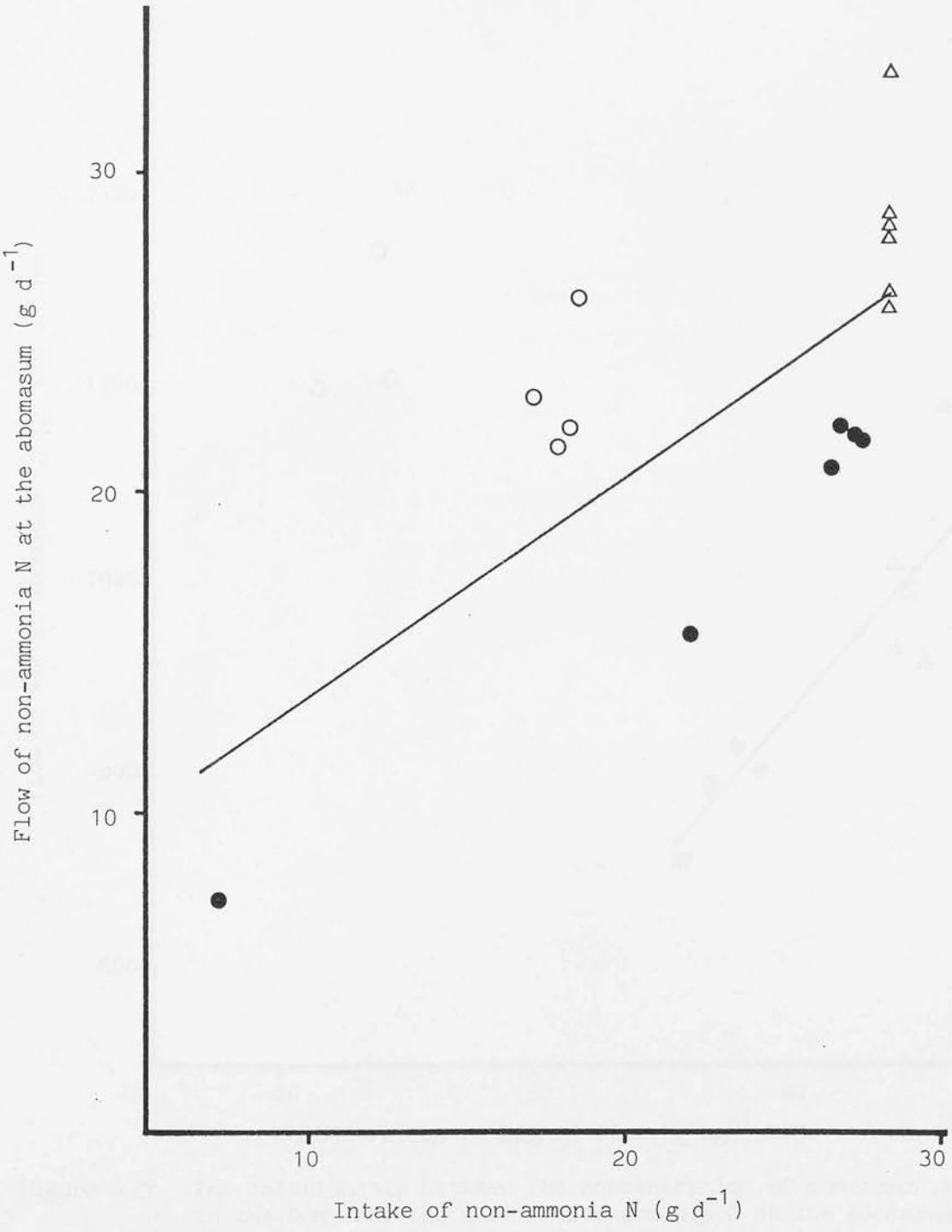

Figure 4.2 The relationship between the intake of non-ammonia N, and the flow of that nutrient at the abomasum. Silage A (○), Silage B (●) and dried grass (△). Line of best fit: $y = 0.697x + 6.904$, $r = 0.723$, $n = 16$

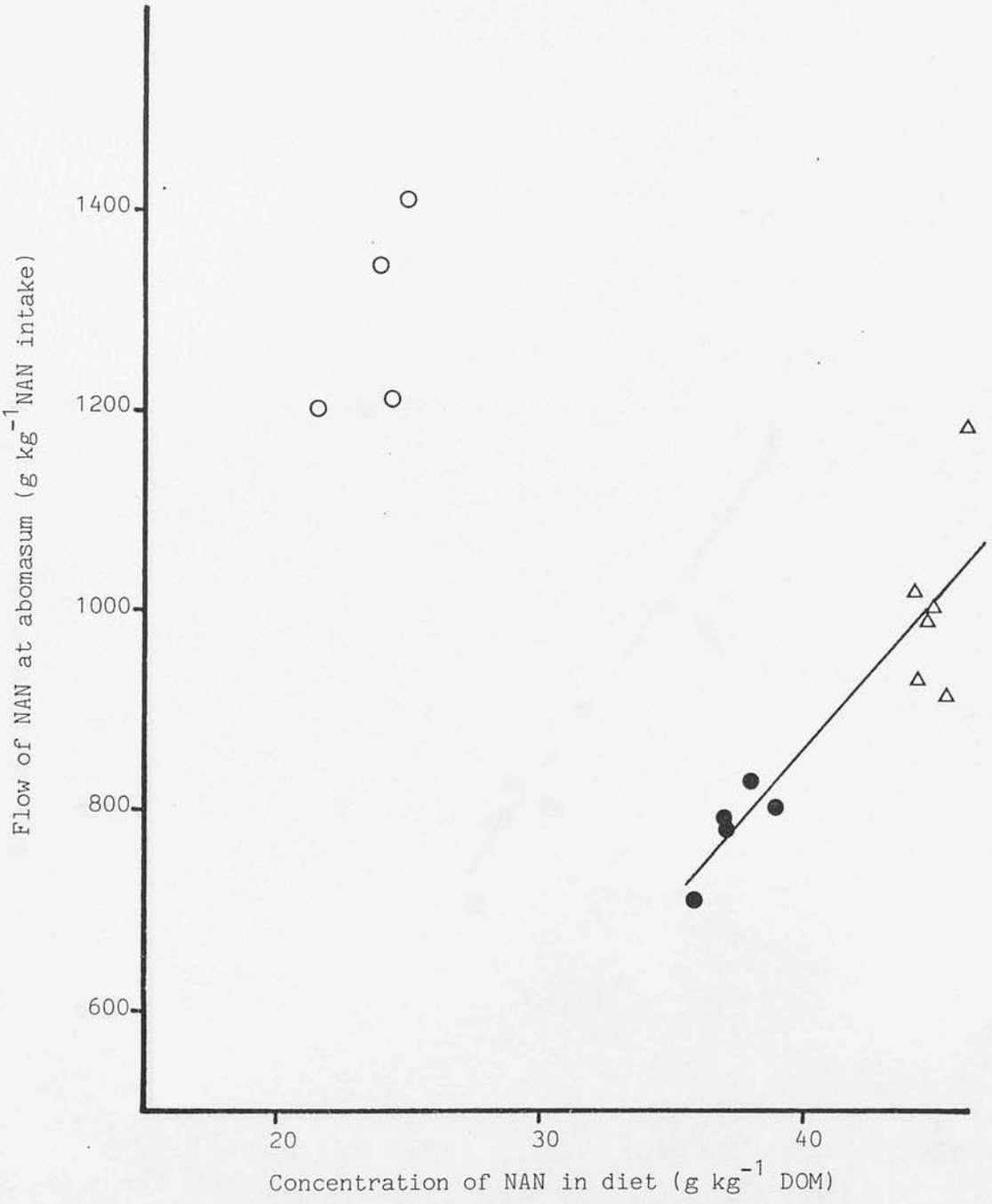

Figure 4.3 The relationship between the concentration of non-ammonia N in the DOM, and the flow of non-ammonia N at the abomasum. Silage A (○), Silage B (●) and dried grass (△). Line of best fit (Silage B and dried grass only): $y = 30.640x - 359.140$, $r = 0.891$, $n = 11$

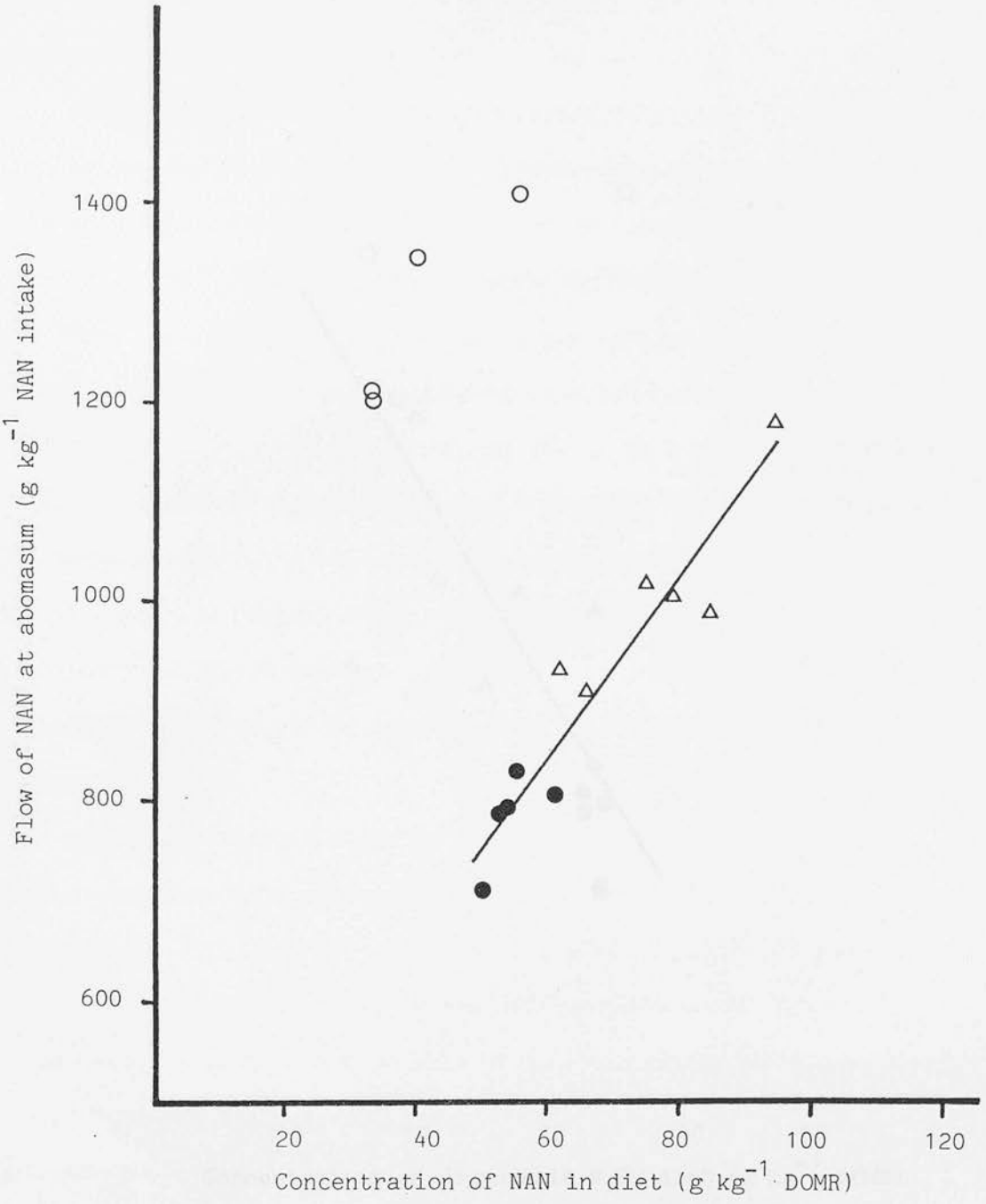

Figure 4.4 The relationship between the concentration of non-ammonia N in the DOMR and the flow of non-ammonia N at the abomasum. Silage A (○), Silage B (●) and dried grass (△). Line of best fit (Silage B and dried grass only): $y = 8.978x + 314.216$, $r = 0.949$, $n = 11$

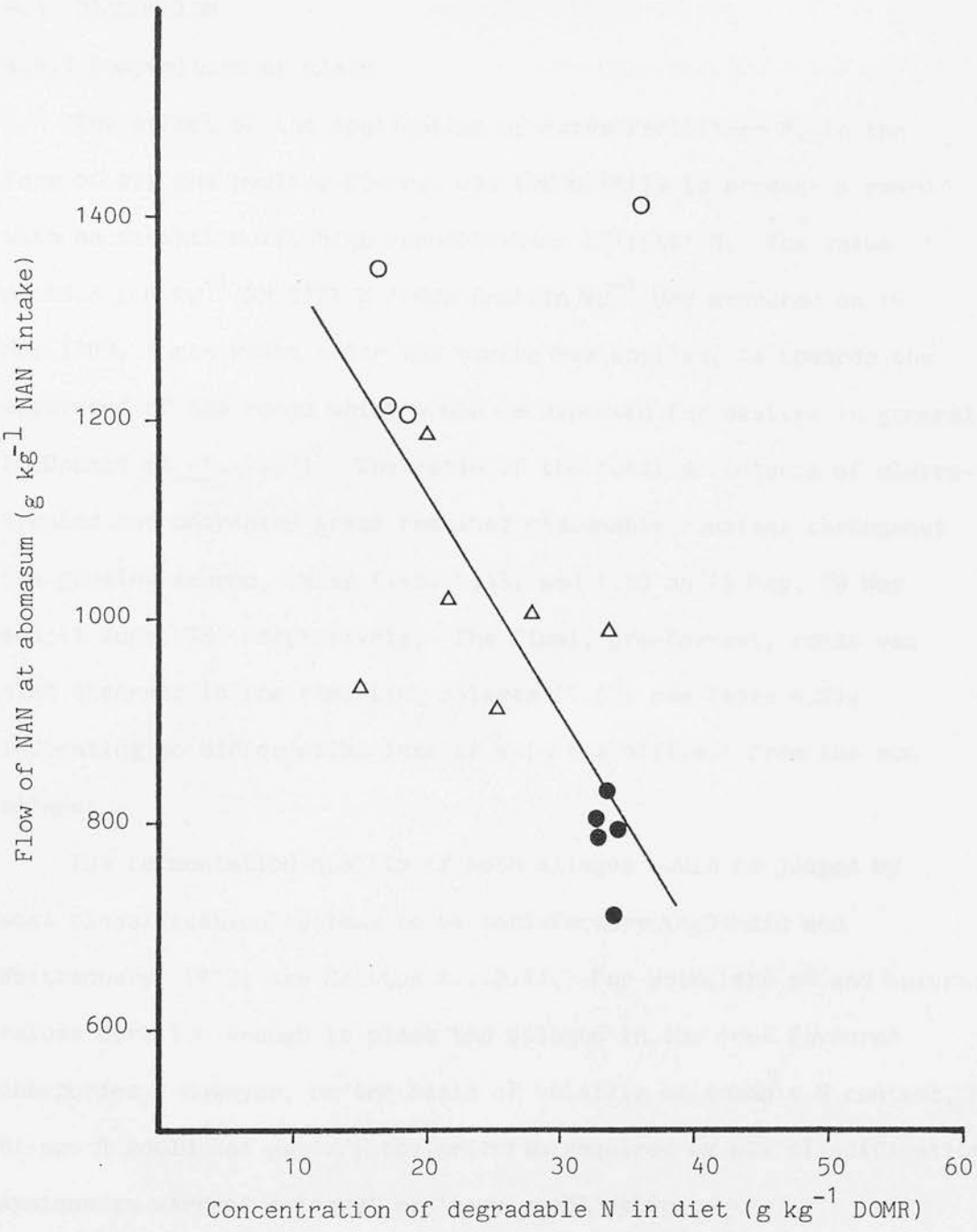

Figure 4.5 The relationship between the concentration of degradable N in the DOMR, and the flow of NAN at the abomasum. Silage A (○), Silage B (●) and dried grass (△).
Line of best fit: $y = -20.502x + 1513.293$,
$r = 0.799$, $n = 14$

4.4 DISCUSSION

4.4.1 Composition of diets

The effect of the application of extra fertiliser N, in the form of pig and poultry slurry, was transiently to produce a sward with an exceptionally high concentration of total N. The value of 43.3 g N kg^{-1} DM (271 g crude protein kg^{-1} DM) measured on 15 May 1980, three weeks after the manure was applied, is towards the upper end of the range which would be expected for pasture in general (McDonald et al, 1973). The ratio of the total N contents of slurry-treated and untreated grass remained reasonably constant throughout the growing season, being 1.48, 1.33, and 1.59 on 15 May, 29 May and 11 June 1980 respectively. The final, pre-harvest, ratio was also observed in the resulting silages (1.57, see Table 4.2), indicating no differential loss of N in the effluent from the two silages.

The fermentation quality of both silages would be judged by most classification systems to be satisfactory (McDonald and Whittenbury, 1973; see Section 2.1.2.4). For both, the pH and butyrate values were low enough to place the silages in the most favoured categories. However, on the basis of volatile or ammonia N content, Silage B would not satisfy the criteria required by all classification systems to warrant a 'good' or 'very good' rating.

In many respects Silage A and Silage B were very similar in composition thus, in part, achieving the goal of producing two materials which differed only in their content of total N. It would, however, have been unrealistic to expect the provision of large quantities of essential nutrients to affect the growth of the sward in a simple manner, and other differences in the composition of the

silages did result. Silage B was appreciably wetter, probably as a result of the higher proportion of succulent leaf material in the harvested sward. The lower water-soluble carbohydrate content of the slurry-treated material would have occurred because of the higher protein content of that material, and the inverse relationship which exists between protein and storage carbohydrate levels in grasses (Simmonds, 1977). Although more water-soluble carbohydrate had apparently been fermented in Silage A than in Silage B (189 vs 167 g kg^{-1} DM ensiled), the higher concentration of these carbohydrates in the untreated grass meant that an appreciable amount of residual water-soluble carbohydrate (18% of that ensiled) was present in the low N silage.

The pelleted dried grass (Table 4.3) had a relatively high content of total N, and a lower level of ammonia N than would be expected in fresh grass (McDonald, 1980). The proportion of the total N which was present as protein N (0.82) was lower than has been reported for fresh or rapidly wilted grass (McDonald, 1980). Proteolysis may have occurred in the period between cutting of the grass and drying it artificially.

4.4.2 Dietary digestibility

The intakes of dry matter achieved for the silages during the measurement of dietary digestibility were high. The intention had been to produce identical intakes of 1 kg DM d^{-1} for all diets, and although there was an apparent trend for the intake of Silage B to be lower than that for the other foods, this was not significant ($P > 0.05$). The mean intake of dry matter of Silage B, 0.863 kg d^{-1} (43.1 g $kg^{-0.75}$ LW d^{-1}), was slightly below the maximum of 0.916 kg d^{-1} (46 g $kg^{-0.75}$ LW d^{-1}) predicted by the Agricultural

Research Council (ARC, 1980) for growing sheep of the same weight. The intake obtained with Silage A was, at 1.025 kg DM d^{-1} (50.9 g $kg^{-0.75}$ LW d^{-1}), greater than the theoretical appetite of 54 kg growing sheep eating silage.

High intakes of silage dry matter have been observed previously, and Morgan et al (1980b) reported intakes of 70.7 and 69.1 g $kg^{-0.75}$ LW d^{-1} for intact Suffolk-cross wethers fed, respectively, unwilted and wilted ryegrass silages. They also observed intakes in excess of theoretical appetites for dry matter, for the same silages offered to fistulated steers. In other studies, however, ruminants have frequently been offered silages in such amounts that the theoretical maximum intakes were not achieved.

The use of data obtained with sub-maximum intakes of dry matter is one of the many areas in which the biological models used to investigate digestive physiology in ruminants are different from the growing or lactating animals to which the results must ultimately be applied (Oldham and Tamminga, 1980).

Gill et al (1979), restricted the intake of silage by wethers to a 'near maintenance' level of approximately 40 g $kg^{-0.75}$ LW d^{-1} and 38 g $kg^{-0.75}$ LW d^{-1} for untreated and formaldehyde-treated silages respectively. Similarly in an experiment in which the nutritive value of silages made from wilted and unwilted grass was compared with that of the fresh grass from which they were made, Donaldson and Edwards (1976) observed a mean intake of 8.5 g kg^{-1} LW d^{-1} for the untreated silage when fed to fistulated wethers. This was less than half of the rate of intake observed in the current experiment, and represented a severe restriction of the animals' theoretical dry matter appetite. Morgan et al (1980a) could only obtain an

intake of 36.0 g $kg^{-0.75}$ LW d^{-1} when offering an unwilted silage of low N content (dry matter = 181 g kg^{-1}, total N = 16.0 g kg^{-1} DM) ad libitum. When feeding silage made from young digestible ryegrass, with a high content of total N (30.5 g kg^{-1} DM), Donaldson and Edwards (1980) observed an ad libitum intake of dry matter of 36.0 g $kg^{-0.75}$ LW d^{-1}. They suggested that the low intake could, in part, be explained by the levels of volatile N and fermentation acids, which were higher than those in silages treated with formalin, or formalin and acids, which were consumed in greater amounts. Nevertheless, the volatile N and fermentation acid levels were no higher for the untreated silage of Donaldson and Edwards (1980) than for either of the silages fed in Experiment One.

There were no differences between the silages in the digestibilities of their dry matter, gross energy or organic matter. If the application of slurry had caused an appreciable difference in the leaf:stem ratio of the sward, or in the growth stage at the time of harvesting, an increase in the digestibility coefficients for these nutrients would have been expected in Silage B (MAFF, 1977). In contrast, the apparent digestibility of the total N in this silage was significantly ($P < 0.01$) higher than that of silage A. This may be explained by the relatively constant contribution of endogenous N to total N in the faeces. This "metabolic faecal N" will reduce the apparent digestibility of N in a food with a low content of total N compared with a similar diet with a higher concentration of the nutrient. The digestibility of total N in silage A was lower than that of dry matter, gross energy or organic matter. A similar trend has been reported for

fresh and wilted silages of moderate N content (Morgan et al, 1980b) and for fresh silage of low total N content (Morgan et al, 1980b). Gill et al (1979) reported similarly low apparent digestion of N in sheep offered an untreated grass/clover silage (0.676), but did not quote the digestibilities of other dietary nutrients. Donaldson and Edwards (1980) found that the total N, dry matter and organic matter in ryegrass silage were all digested to the same extent by fistulated sheep. Although no values for dried or unpelleted material are available for direct comparison, the low digestibility coefficients observed for the pelleted, dried grass were expected. Beever et al (1972) reported that the digestibility of dry matter, organic matter and gross energy were all lower for ground, pelleted dried grass than for chopped material.

The concentration of digestible and metabolisable nutrients in the diets, as shown in Table 4.6, follow the pattern which would be expected from their digestibility coefficients and their concentrations in the three diets. Thus, the silages did not differ in their DOMD, DE or ME values whereas the digestible crude protein (DCP) content, being the resultant of digestibility and concentration, was almost 80% higher for Silage B than for Silage A. This difference was highly significant ($P < 0.001$) and meant that the nutritive value of the high N silage would be assessed as being much greater than that of the low N silage, in any rationing system based on the provision of digestible nutrients (MAFF, 1976; Herland, 1982; Poppe and Gabel, 1982; Rohr, 1982).

The nutrients in silage are often considered to be inefficiently utilised with the result that urinary excretions of N and gross energy, as a proportion of their intakes, are higher than in other

classes of food (McDonald and Edwards, 1976). In the present experiment, significant differences were observed between the silages, with more of the total N and gross energy of the high N silage being excreted in the urine. Since the apparent retention of N in the animal's body was the same for both silages (Table 4.7), it might be assumed that the higher excretion merely reflected the provision of a nutrient in excess of the requirements of an animal with a very limited productive capacity. Against this, however, must be set the observation that the sheep, although nominally mature, still had the potential for retaining significantly more N than was achieved with either of the silage diets; the value of 7.28 g N retained d^{-1} obtained with the dried grass being significantly higher ($P < 0.05$). The absolute values of the energy losses in urine are low compared with some previous observations. Donaldson and Edwards (1980) found that 8.9% of the gross energy of an untreated silage fed to fistulated wethers was excreted in the urine.

4.4.3 Rate of flow of digesta

The intakes of dry matter during the period when flow rates were measured were essentially the same as those recorded earlier and were, for the silages, close to the maximum predicted for the class of animal and type of diet (ARC, 1980).

For a material to function satisfactorily as a nutritional marker it must be fully recoverable from the material it is to mark (Kotb and Luckey, 1972). In the context of markers for the indirect measurement of the rate of flow of digesta, this means that the marker must not be absorbed from the gastrointestinal tract, between the point at which it is administered and the point

at which the digesta sample is taken. However, markers are used to estimate rates of flow indirectly in animals fitted with T-piece cannulas, which preclude total sampling, and hence estimation of the recovery of the marker must be made at a point further down the digestive tract. In this experiment, as in most others, the recoverability of the markers was assessed in the faeces, thus providing an assessment of losses between the rumen and the anus. Recovery of Cr was slightly higher than that of Ru, but for both markers the recovery, which showed no significant variation attributable to diet, was lower in absolute terms than has been reported elsewhere. Macrae and Evans (1973) observed recoveries of 96% and 101% for Ru in the faeces of two sheep given continuous intraruminal infusions of Ru-P for 9 d, and a mean recovery of 101% for 24 sheep, each given a single dose of the same marker. Beever et al (1978) reported recoveries of 99.5 $\pm$ 2.32% and 100.4 $\pm$ 0.45% for Ru-P and Cr-EDTA respectively, in an experiment in which the effects of infused markers on the digestion of dry matter by intact sheep, were investigated.

It is difficult, given the available data, to explain adequately low recoveries of Ru and Cr observed in Experiment One. Since this was the first experiment involving continuous infusion of markers for the measurement of flow rate, which had been conducted in the Department, technical problems were inevitable. The continuity of the 'continuous' infusion was poorer in the early stages of the experiment because of occlusion of the transmission tubing with particulate ruminal digesta or owing to tight coiling of the tube within the sheep's rumen. These problems were overcome by ensuring that fluid was pumped at all times when the tubing was

connected to the animal, and by limiting the extent to which the tube protruded into the rumen to approximately 10 cm. Because of these early problems, differences between periods were significant ($P < 0.05$) for the recovery of Cr, and nearly so for Ru, for which the period mean square was the largest variance term. Recoveries of Ru and Cr were, however, low in all experiments (see Sections 5.3, 6.3 and 7.3) and possible reasons for these observations are discussed in Section 8 below.

The reconstitution factors shown in Table 4.9 illustrate the importance of using a dual phase marker system to counteract biased sampling when using a simple T-piece cannula. In the absence of sampling bias, the concentrations of Ru and Cr in sampled and centrifuged digesta would be such that the value of the reconstitution factor was zero. No correction would then need to be applied to the observed concentrations of Ru and Cr in sampled digesta and the rate of flow could be calculated from either of these observed concentrations and the corresponding infusion rate for the marker (see Appendix A). Since all the reconstitution factors are negative, centrifuged digesta had, in effect, to be 'withdrawn' from the sampled material to reconstruct accurately the material flowing past the point of sampling. Thus, in the original sample, solids, and consequently Ru, were present in disproportionately high concentrations. A similar sampling bias has been reported by Faichney (1975b) whereas Beever et al (1978), working with fistulated calves, observed the same bias at low rates of flow but the opposite effect, with less Ru than was expected, when the rate of flow of digesta was high.

No significant differences, attributable to silage type, were observed between the rates of flow of dry matter or organic matter at the abomasum. In view of the known effects of drying, grinding and pelleting grass on the partition of digestion within the digestive tract of ruminant animals (Beever et al, 1972), the relatively high rates of flow of dry matter and organic matter observed for the dried grass were expected. However, the high random variability associated with these measurements (cv% = 35.9 and 43.9 for dry matter and organic matter flow respectively) meant that only the difference between the dry matter flows for dried grass and Silage B was statistically significant ($P < 0.05$).

No significant differences were demonstrated between the rates of flow of total N or non-ammonia N for the silages despite the fact that the intakes of both nutrients were higher for Silage B. Silage B had a much higher level of DCP and consequently, in the view of certain rationing systems, a greater potential to supply nitrogenous nutrients. It must be accepted that total N flow at the abomasum may be a poor indicator of the supply of amino acids to the absorptive regions of the gastrointestinal tract. However, non-ammonia N has been advocated as a readily measurable parameter which is likely to be closely correlated with the level of amino N which in turn is a reasonable predictor of the value, to the animal, of the nitrogenous compounds presented at the start of the small intestine (Oldham and Tamminga, 1980). It thus appears that Silage B was in no way superior to the low N material in terms of its ability to supply nitrogenous nutrients and that the efficiency with which non-ammonia N was transferred from the diet to the small intestine was much lower for the silage with the high DCP content.

This is illustrated very clearly in Table 4.11 where nutrient flows are expressed in terms of their intakes. These values show that there was an apparent gain of total N and non-ammonia N between the mouth and the abomasum for Silage A and a loss over the same section of the tract when Silage B was fed.

An apparent loss of total N between the mouth and abomasum or small intestine has been reported for silages with a high content of total N by P.C. Thomas (pers. comm.). He concluded that, for silages made without the application of a formaldehyde-containing additive, a net loss of N across the rumen occurred consistently at crude protein levels above 160 g kg^{-1} DM (total N level of 25.6 g kg^{-1} DM). The crude protein content of Silage B in the present experiment (185 g kg^{-1} DM), unfortunately places it between two of six categories described by Thomas, but the observed N flow:N intake ratio (0.808) is also intermediate (0.96 for crude protein = 160-170 g kg^{-1} DM and 0.75 for crude protein = 190-200 g kg^{-1} DM).

At low levels of dietary N, Thomas (pers. comm.) found that apparent N flow was greater than N intake, although none of the diets they included in their survey had as low a crude protein content as the 118 g kg^{-1} DM of the low N silage in this experiment. When feeding three semi-purified rations with total N contents of 9.29, 7.77 and 7.93 g kg^{-1} DM, Pisulewski, Okorie, Buttery, Haresign and Lewis (1981) frequently observed values greater than unity for the ratio of N passage at the duodenum to N intake. Not all experimenters have observed this phenomenum. Crickenberger et al (1973) offered silages of low (113 g crude protein kg^{-1} DM) and high (128 g crude protein kg^{-1} DM) protein content to steers

fitted with rumen and abomasal cannulas. Although the ratio of abomasal N passage:N intake was lower for the high than for the low protein silage, both ratios were less than unity indicating an apparent loss of N over the rumen for both diets. Similarly Morgan et al (1980b) measured a net loss of N (abomasal N flow: N intake ratio = 0.90) when ryegrass silage of a very low crude protein (100 g kg^{-1} DM) was fed to fistulated wethers. In this last experiment, however, a dual-phase marker was not employed and the accuracy of the rates of flow of abomasal digesta must be suspect.

Both in absolute terms (Table 4.10), and per unit intake of total N in the diet (Table 4.11), the passage of microbial N at the abomasum was greater for the silage of low, than for the silage of high, N content. Since the rate of flow of total N was similar for these diets, microbial N accounted for a significantly higher ($P < 0.01$) proportion of that total when Silage A was fed (Table 4.12). However in absolute terms these values are low compared with others which have been published. Beever (1980) reported that more than 70% of the duodenal N flow, obtained when a silage was fed to sheep, was microbial in origin, and Beever et al (1977) found that 71% of the amino N reaching the duodenum of sheep fed untreated silage, came from this source.

The values for the degradability of dietary N of all three diets (Table 4.12) are somewhat lower than the averages which have been reported for these classes of food, and are lower also than those suggested by the Agricultural Research Council in their recent review on the protein requirements of ruminant livestock (ARC, 1980). However the variability associated with measurements

of this type is high and dependent on the animals, analytical techniques and feeding regime employed (ARC, 1980; 1984). The coefficients of variation obtained in this study were, at 42% and 32% for uncorrected and corrected degradabilities respectively, high even by the standards expected in animal experiments of this sort. Thus, if a degradability of 0.71 (the lower end of the range proposed by the Agricultural Research Council (1980) for silages) had been observed for a fourth diet in this experiment, it would only just have been distinguishable, statistically, from the value for Silage B. It is encouraging that the silages and the dried grass are ranked in the order which would be expected from previous work (ARC, 1980), and that the extent of the degradation of the nitrogenous fraction of the silages is greater ($P < 0.05$) than that of the dried grass.

The average values for the proportion of digestible organic matter which was apparently digested in the rumen (Table 4.13), are again lower than the suggested universal value of 0.65, which is used in the factorial calculation of rumen degradable N requirements in the system of the Agricultural Research Council (ARC, 1980; 1984). However in this case the values are sufficiently close to those which have been previously observed for grass silages (Beever et al, 1971; ARC, 1980) to encourage belief that they are representative of the overall population of such values. Because more microbial N was produced for the fermentation of no more organic matter, the apparent efficiency of synthesis of microbial N in the rumen was higher ($P < 0.05$) for Silage A than for Silage B. This efficiency has been expressed in numerous ways (see Section 2.4) and the most often quoted, 'g N kg^{-1} DOMR', has now been superseded by 'g N MJ^{-1} ME intake' as the term preferred by the

Agricultural Research Council (ARC, 1980; 1984). None of the values for microbial synthesis per unit DOMR or per unit ME intake reported here, is either outside the range which has been previously reported or appears to deviate from the overall means which have been compiled by the Agricultural Research Council (30 g N kg^{-1} DOMR (ARC, 1980); 32 g N kg^{-1}DOMR, 1.32 g N MJ^{-1} ME intake (ARC, 1984)). The balance between the supply of rumen degradable organic matter and rumen degradable N was different in the two silages, with the ratios of degradable OM:degradable N being 42.3 and 26.9 for Silage A and Silage B respectively. A similar trend was observed by Beever (1980) who found that as the ratio of degraded carbohydrate:degraded N increased from 9.8 to 15.0, the efficiency of synthesis of microbial N, in sheep fed silage diets, increased from 39 to 41 g N kg^{-1} DOMR. However in the same experiment fresh grass, with an intermediate degraded carbohydrate:degraded N ratio of 13.6, supported a more efficient rumen fermentation with a value of 53 g N kg^{-1} DOMR for the synthesis of microbial N.

The figures in Table 4.14 describe, in various ways, the relationship between the nitrogenous nutrients and energy, supplied by the diets. In their recent review (ARC, 1984), the Agricultural Research Council re-analysed published data on the flow of non-ammonia N to the duodenum of ruminant animals in an attempt to produce empirical equations which would relate the flow of the nutrient to the intake of N and ME. They concluded that such a simple approach could only yield a relationship which would have application limited to a single class of diet or species of animal. Oldham and Tamminga (1980) found that non-ammonia N flow per unit intake of ME (g NAN MJ^{-1} ME intake), which is presented in

Table 4.11, varied as a result of changes in the type of diet and consequently in the patterns of rumen fermentation. The proportion of roughage to concentrate affected the value, although not in a consistent manner. The maximum values reported by Oldham and Tamminga were in the range 2.5 - 3.0 g NAN MJ^{-1} ME, and a value of 2.8 was recorded when pelleted, dried grass was fed to dairy cattle (cf. 2.93 for the dried grass fed in the current experiment). In contrast, the values which were recorded for the silages were lower and would, if the figures given in the Agricultural Research Council's review (ARC, 1984) for microbial N synthesis applied (1.32 g N MJ^{-1} ME intake), indicate that at least 80% of the non-ammonia N at the abomasum was microbial in origin. However, it has already been demonstrated that in this experiment the efficiencies of synthesis of microbial N were lower than the figure of 1.32 g N MJ^{-1} ME intake suggested by the Agricultural Research Council and the g non-ammonia N MJ^{-1} ME figures in Table 4.11 therefore include microbial components of only 64% and 41% for Silage A and Silage B respectively.

When examining the results of a large number of experiments conducted with cannulated animals, the Agricultural Research Council (ARC, 1984) identified linear regressions relating the flow of non-ammonia N at the duodenum to the intake of total N, ME or both, for a range of diet types. For silages and dried grasses fed to sheep, non-ammonia N flow was best related to the intake of total N alone. The regression equations were:

$$y = 0.74x + 3.56$$

and

$$y = 1.11x - 0.84$$

for silages and dried grasses respectively, where y is non-ammonia N flow to the duodenum (g d^{-1}) and x is intake of total N (g d^{-1}).

Although the proportion of the observed variation which was accounted for by these regressions was high (0.74 and 0.82 for silages and dried grass respectively), it was concluded that such simple models, which could take no specific account of variations in the extent of microbial manipulation of dietary N, would be unsatisfactory. Thomas (1982) concluded that a significant positive relationship existed between the intake of total N in the diet and flow of that nutrient at the duodenum. However, the line of best fit for the data described was, over the range of most of the observations, very close to the line of equality (x = y). This indicates that the concentrations of total N in the diets selected by Thomas were relatively constant and did not include any extremes of high or low N content, for which the rates of flow would have been expected to be, respectively, markedly lower and higher than N intakes.

In Figures 4.2 - 4.5, several different relationships between the intake of nitrogenous nutrients, or the concentrations of these nutrients in the diet and the supply of non-ammonia N to the abomasum, are presented. For the simple relationship between the intake and flow of non-ammonia N (Fig 4.2), it can be seen that a statistically significant positive relationship does exist. However, the fact that a straight line can be fitted to a set of observations does not mean that it is necessarily a sensible interpretation of the data or that it has any meaningful predictive value. Thus the fitted line does not adequately describe the relationship that exists between the two silages since, as is evident from Table 4.11,

the specific supply of non-ammonia N is much less from the high N than from the low N silage.

Figures 4.3 and 4.4 illustrate a problem which can arise when attempting to fit general relationships to data with an uneven distribution. In both, a negative linear relationship could be established for the silage diets - but only for the same reasons that a straight line can always be drawn between two points. In neither figure can a linear relationship be used to describe the values of all three diets. In both, however, as the ratio of non-ammonia N to the organic matter component increases, the 'efficiency' with which dietary non-ammonia N is supplied to the abomasum by the high N silage and the dried grass increases in the same manner. There is a suggestion that a similar positive relationship, displaced by about 600 - 800 units on the y-axis, may exist for the low N silage. However, the limited number of observations and their restricted range make the value of such a relationship doubtful.

In Figure 4.5, the parameter plotted on the x-axis is intended to index the ability of the diets to promote the synthesis of microbial N in the rumen. When all the values are included, the negative relationship obtained is poor. However after observing the scatter diagram, it would not seem unreasonable to exclude the highest value for the low N silage which alone does not seem to belong to a common data group. The remaining values do demonstrate a significant negative relationship which, for this experiment, seems to be applicable to all diet types.

4.5 CONCLUSIONS

The results from this experiment demonstrate that supplementary manuring of a mixed grass sward can increase the level of total N in the harvested grass. Silages resulting from the ensiling of ryegrass swards of two different levels of total N are likely to differ, by a similar amount, in their contents of N. They can, even in the absence of additive or field-drying treatments before ensiling, be well preserved. The dry matter intake of such silages by mature, cannulated, wethers may be equal to, or greater than, the intake predicted for growing sheep of similar weight.

Since the apparent digestibility of total N tends, because of the constant contribution of metabolic N to total N in the faeces, to be higher in a silage with a high content of the component, such a silage will have a markedly higher content of DCP than a similar silage with a low content of total N. Silages differing by as much as 80% in their content of DCP may, when consumed by sheep in similar amounts, supply almost identical quantities of nitrogenous nutrient to the small intestine. This means that systems for rationing ruminant animals, which rely on estimates of the provision of digestible nutrients by different foods, are likely to prove inaccurate.

It is difficult to produce empirical relationships, relating the supply of nitrogenous nutrients to the composition of foodstuffs, which are applicable to a range of diets. In the present studies the supply of non-ammonia N was inversely related to the content of degradable N in the diets' ruminally degraded organic matter. Since the last two terms describe, respectively, the supply of N and energy for rumen microbial growth, it appears that the extent

or efficiency of this process has a major influence on the nitrogenous nutrition of the sheep.

5. EXPERIMENT TWO

5.1 INTRODUCTION

Formic acid is the most commonly used pre-ensiling additive in Europe (Henderson and McDonald, 1976; Crawshaw, 1977; Drysdale, 1979). As 'Add-F' (BP Nutrition (UK) Ltd), it is widely used when ensiling unwilted grass silage (Adamson and Appleton, 1984) and, because of its potent antimicrobial activity, it has been used to preserve more unusual foods. Thus Patterson and Walker (1981) used the acid to preserve effluent from grass silage, and Strøm and Eggum (1981) added a mixture of formic and propionic acids to minced fish viscera before ensiling.

Formic acid is mainly used with crops which would be difficult to ensile successfully when untreated, because of low dry matter or water-soluble carbohydrate contents (Henderson and McDonald, 1976; Adamson and Appleton, 1984). The effects of formic acid on the composition of silage are well documented, and include reduced production of ammonia and less degradation of herbage nitrogenous compounds and water-soluble carbohydrates (Henderson and McDonald, 1976; Hinks and Henderson, 1977; Barry et al, 1978a; McDonald, Proven and Henderson, 1983).

A few workers have also reported direct effects of formic acid on the nutritive value of silage. These are frequently beneficial, and Siddons, Beever and Kaiser (1982) and McDonald et al (1983) both observed slight reductions in the degradation in vitro and in sacco of nitrogenous compounds in formic acid-treated silages. Barry et al (1978a) noted increased retention of N when treated silage was fed to sheep, a consequence of increased supply of N to the small intestine. In contrast, Beever

(1980) cited work which suggested that synthesis of bacterial N and the supply of N to the small intestine were both less efficient with formic acid-treated than with untreated silage.

In the present study, the effects of formic acid, applied at a commercial rate, on the composition of ryegrass silage and on the nutritive value of that silage when fed to sheep, were investigated.

5.2 METHODS AND MATERIALS

5.2.1 Experimental design and procedure

The animal studies of Experiment Two were conducted between 25 January and 23 May 1982. The three experimental periods were each of five weeks' duration and the second and third followed the format described in Section 3.2 above. Sheep were kept in metabolism cages throughout Period One, but it was apparent that continuous confinement was distressing to some of the sheep, which exhibited stereotyped behaviour (Woodgush, Duncan and Fraser, 1975) and reduced intake of food. Problems were also experienced in obtaining samples of abomasal digesta from two animals. Because of these difficulties, the start of the second period was delayed for two weeks while replacement animals were introduced to the test diets. The manner in which the animals were restrained was also altered to that described in Section 3.2, thus minimising the amount of time spent in the metabolism cages.

Eight sheep (numbers 74, 91, 135, 155, 218, 487, 1190 and 1280) were used. The average weight of these animals over the course of the experiment was 59.0 kg, with a minimum of 44.0 and a maximum of 73.6 kg. The allocation of diets for each animal is shown in Figure 5.1.

	Column					
	I	II	III	IV	V	VI
Sheep	218	1280/155	91/1190	1190/74	135	487
Period						
1	D	C	DG	D	C	DG
2	DG	D	C	C	DG	D
3	C	DG	D	DG	D	C

Figure 5.1 Allocation of three diets, dried grass (DG), silage with no pre-ensiling treatment (C) and silage treated with formic acid before ensiling (D), to eight sheep over three experimental periods in Experiment Two

As in Experiment One, the intended schedule for the experiment was disrupted. The deviations from the intended routine were as follows:

(i) In Period One, Sheep 1280 exhibited signs of stress, apparently associated with confinement. Voluntary intake of food was low and the animal displayed characteristic stereotyped behaviour, standing for long periods of time with its head and shoulders forced through the front feeding aperture of the cage and resisting all reasonable attempts to move it back inside. The sheep was consequently replaced by number 155 for the second and third periods.

(ii) The intake of food by Sheep 1190 in Period One was low (7.8 g DM kg^{-1} liveweight). Perhaps as a result of this, no abomasal digesta could be collected through the cannula during the fifth week of the period. Thus, no values were obtained for any of the parameters of digesta flow rate for this animal (Column IV, Period One). Because of the risk of continued dysfunction, Sheep 1190 was replaced by

Sheep 74 for the second and third periods.

(iii) The intake of Silage C by Sheep 91 in Period Two was so low that the measurements made during the digestibility and rumen metabolism phases were considered unrepresentative. These figures were therefore treated as missing values in the statistical analysis. Only a very small sample (<200 ml) of abomasal digesta was obtained from the sheep and this was insufficient to conduct all the required analyses. Thus only flows of dry matter and total N were determined. These also were considered unrepresentative and were not used. In Period Three, the place of Sheep 91 was taken by Sheep 1190, from which abomasal digesta could, by this time, be readily obtained.

(iv) No abomasal digesta could be obtained from Sheep 135 in Period Three. Thus, no parameters of digesta flow rate were available for this animal (Column V, Period Three).

5.2.2 Diets

Three diets were offered in Experiment Two, two grass silages and the pelleted, dried grass which was fed in each experiment.

5.2.2.1 Silages

The herbage for both silages was harvested from a mixed ley of Italian and Perennial ryegrass and Annual Meadow-grass (Poa annua). The Italian and Perennial ryegrass constituted, respectively, 46 and 53% of the sward, and both species were at full ear emergence when cut on 15 June 1981. The area had received 110 kg N ha^{-1} (as ammonium nitrate) on 13 April 1981, and indeterminate applications of pig and poultry slurry during the Spring.

The crop was cut with a drum mower and lifted immediately using a precision-chop forage harvester. About 4 t of harvested material was ensiled, untreated, in a concrete bunker silo 1.6 m wide, 2.8 m long and 1.6 m deep. The grass was consolidated manually as the silo was being filled, and when this was completed, the ensiled material was covered with polythene sheeting which was sealed, and weighted with sandbags. The remainder of the grass from the field was ensiled in a walled clamp silo of approximately 100 t capacity. The grass was treated with formic acid, applied as an 85% solution (Add-F, BP Nutrition Ltd, 3.4 l commercial product t^{-1} ensiled) through an applicator mounted on the forage harvester. The position in this silo, of the grass harvested from the area adjacent to that from which the grass ensiled in the bunker silo was cut, was noted.

The bunker silo was opened on the 14 October, and the clamp silo on 15 October 1981, after ensiling periods of 121 and 122 d respectively. For each, surface spoilage was removed and approximately 1.5 t of the silage (Silage C from the bunker silo and Silage D from the larger clamp silo) was placed on a clean concrete floor and thoroughly mixed. The bulk was then representatively sampled for subsequent analysis (see Section 3.4.1), and 210 polythene bags were each filled with 6 kg of silage. This was slightly in excess of the amount required to provide the daily ration of 1 kg dry matter. The bags were sealed and then stored at -20^{o}C until required. The silages were thus in refrigerated storage for between 100 and 220 d before being thawed and fed to the sheep.

5.2.2.2 Dried grass

The pelleted, dried grass was stored in a pest-proof container. At intervals during the course of the experiment, pellets were weighed into polythene bags to provide a daily ration of 1.14 kg (1 kg dry matter).

5.3 RESULTS

5.3.1 Composition of diets

The mean composition of representative samples taken from the standing crop the day before it was harvested, and from the grass which was ensiled, are presented in Table 5.1. The compositions of the resulting silages are shown in Table 5.2. It is noteworthy that although care was taken to ensure that similar material was ensiled in both silos, the silages had markedly different contents of total N, a parameter which is unlikely to have been influenced by the pre-ensiling additive.

The composition of the pelleted, dried grass is shown in Table 5.3.

Table 5.1 Composition of the standing crop and the herbage ensiled

	Standing Crop	Grass as ensiled	
		Silage C	Silage D
Dry matter (g kg^{-1})	196	183	186
Water-soluble carbohydrate (g kg^{-1} DM)	316	227	227
MAD fibre (g kg^{-1} DM)	268	-	-
Gross energy (MJ kg^{-1} DM)	19.2	18.2	18.2
Total N (g kg^{-1} DM)	16.2	13.1	16.9

Table 5.2 Composition of silages

Component (g kg^{-1} DM unless otherwise stated)	Silage C	Silage D
Dry matter [1] (g kg^{-1})	193	173
Dry matter [2] (g kg^{-1})	199	190
pH (units)	3.65	3.84
Organic matter	926	924
Water-soluble carbohydrate	0	8
Ethanol	25	40
Lactic acid	126	89
Acetic acid	31	34
Propionic acid	0.4	0.9
N-butyric acid	0.6	0.7
Formic acid	1.0	9.6
Total N	14.0	17.0
Protein N	4.3	6.5
Protein N (g kg^{-1} total N)	306	383
Ammonia N	1.0	1.2
Ammonia N (g kg^{-1} total N)	72	71
Non-ammonia N	13.0	15.8
Non ammonia N (g kg^{-1} total N)	929	929
Gross energy (MJ kg^{-1} DM)	18.6	19.6

1 Dry matter determined by drying to constant weight at 100°C

2 Dry matter determined by distillation with toluene (see Section 3.4)

Table 5.3 Composition of pelleted, dried grass

Component (g kg^{-1} DM unless otherwise stated)	Pelleted, dried grass
Dry matter [1]	878.0
Organic matter	933
Water-soluble carbohydrate	190
Total N	28.5
Protein N	23.4
Protein N (g kg^{-1} total N)	821
Ammonia N	0.3
Ammonia N (g kg^{-1} total N)	11
Non-ammonia N	28.2
Non-ammonia N (g kg^{-1} total N)	989
Gross energy (MJ kg^{-1} DM)	18.8

1 Determined by drying to constant weight at $100^{0}C$

5.3.2 Ruminal metabolism

Diurnal variation in the concentration of ammonia N and in the pH, of strained rumen liquor are presented in Tables 5.4 and 5.5 respectively. These parameters are illustrated graphically in Fig 5.2 and 5.3. There were few significant differences between the silages in either the pH or the ammonia N content of the rumen liquor at any given sampling time. The non-glucogenic ratio (NGR) of the mixture of volatile fatty acids in strained rumen liquor, is plotted against time in Fig 5.4. The NGR was calculated as:

$$\frac{\text{(acetate + 2 butyrate + valerate)}}{\text{(propionate + valerate}}$$

using acid concentrations expressed in mmol l^{-1}, and being a measure of the ratio of non-glucogenic to glucogenic precursors.

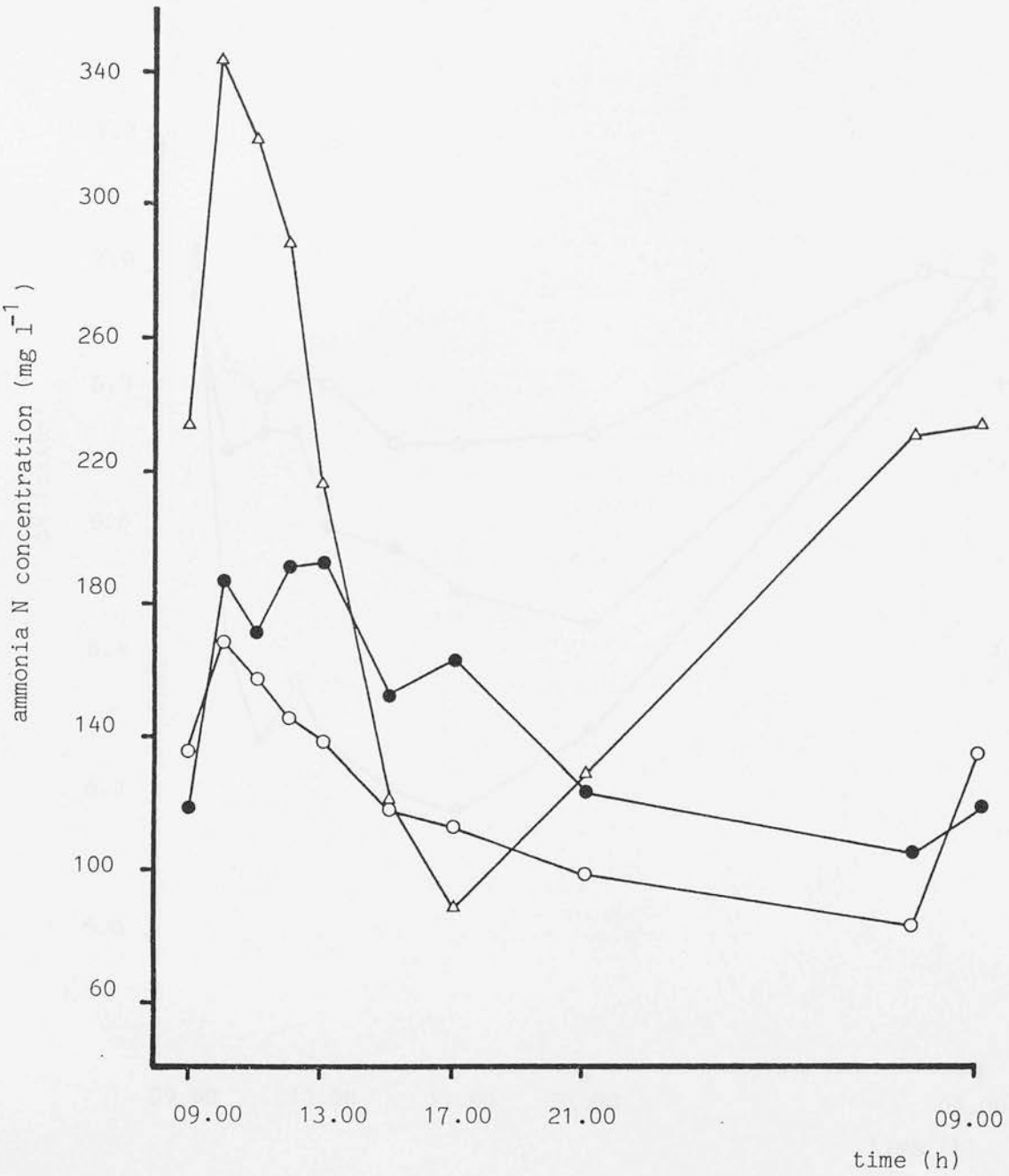

Figure 5.2 Diurnal variation in the concentration of ammonia N in the strained rumen liquor of sheep fed once daily at 09.00 h.
Silage C (○), Silage D (●), dried grass (△)

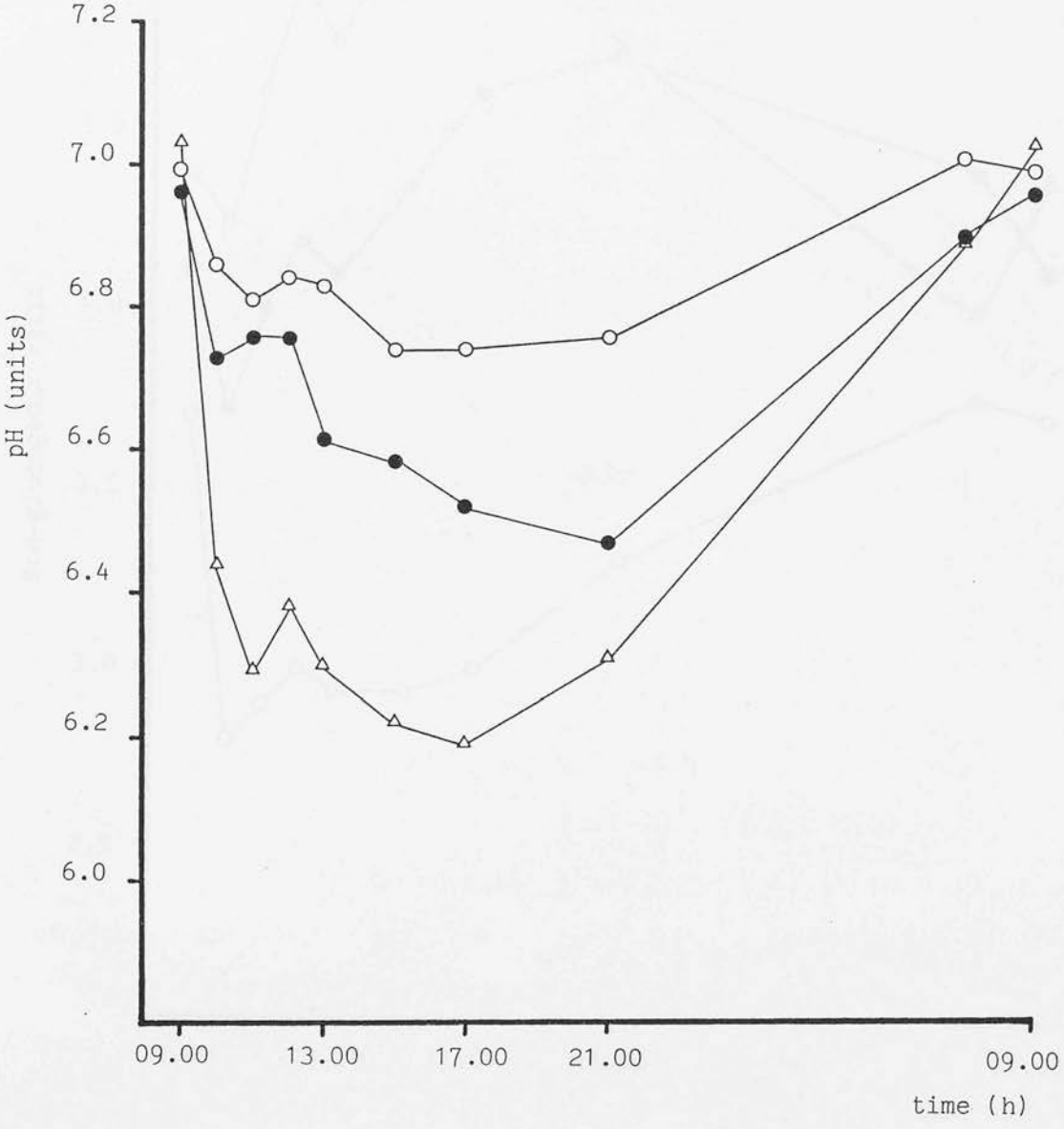

Figure 5.3 Diurnal variation in the pH of the rumen liquor of sheep fed once daily at 09.00 h. Silage C (○), Silage D (●), dried grass (△)

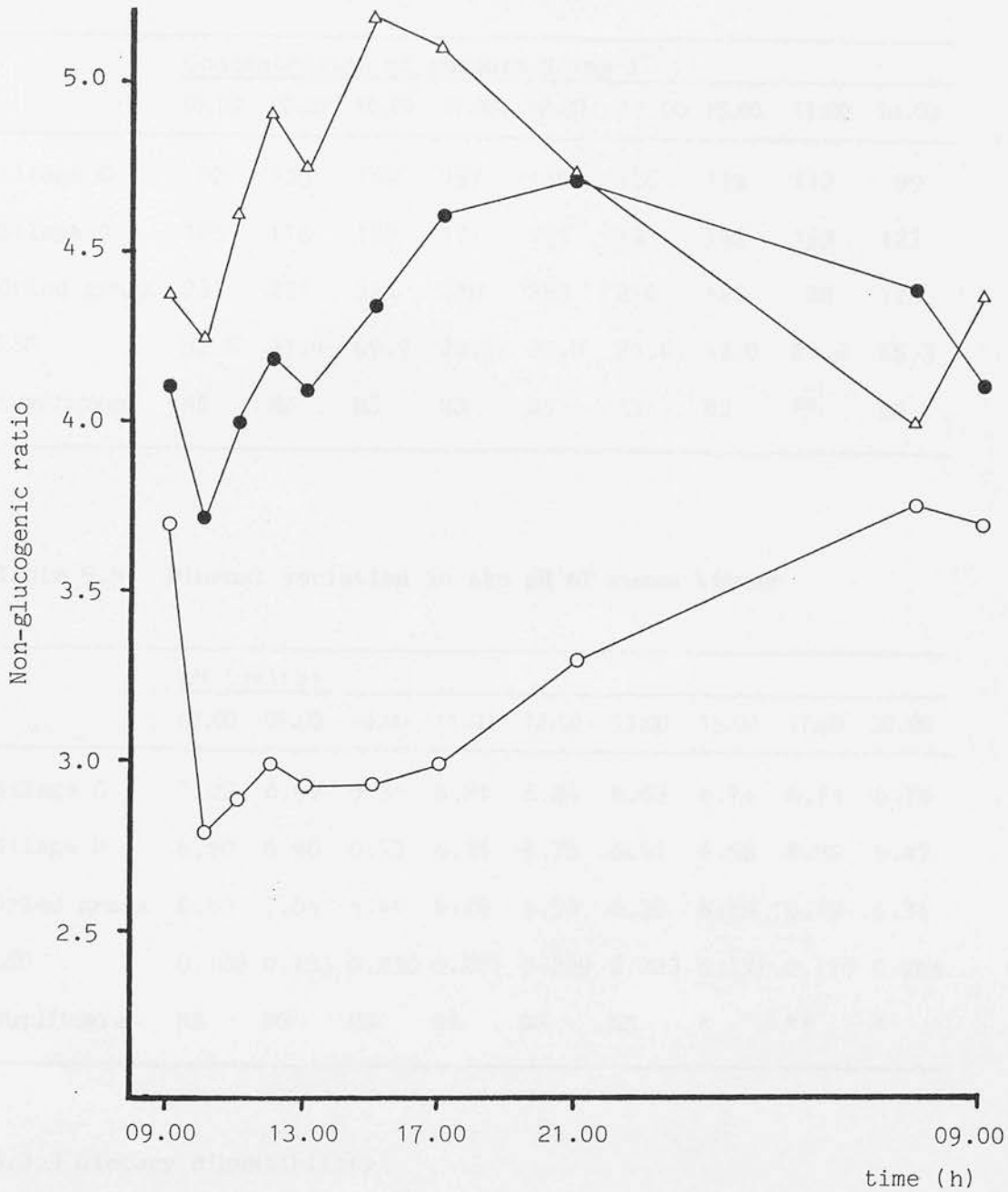

Figure 5.4 Diurnal variation in the non-glucogenic ratio of the volatile fatty acids in strained rumen liquor of sheep fed once daily at 09.00 h. Silage C (○), Silage D (●), dried grass (△)

Table 5.4 Diurnal variation in the concentration of ammonia N in strained rumen liquor

	Concentration of ammonia N (mg l^{-1})								
	07.00	09.00	10.00	11.00	12.00	13.00	15.00	17.00	21.00
Silage C	82	135	169	157	145	138	118	113	99
Silage D	105	118	186	171	191	193	152	163	123
Dried grass	231	234	344	320	288	216	121	88	129
LSD	52.8	37.4	69.9	20.7	25.0	86.4	49.0	24.2	65.3
Significance	NS	NS	NS	NS	NS	NS	NS	**	NS

Table 5.5 Diurnal variation in the pH of rumen liquor

	pH (units)								
	07.00	09.00	10.00	11.00	12.00	13.00	15.00	17.00	21.00
Silage C	7.02	6.99	6.86	6.81	6.84	6.83	6.74	6.74	6.76
Silage D	6.90	6.96	6.73	6.76	6.78	6.61	6.58	6.52	6.47
Dried grass	6.89	7.04	6.44	6.29	6.38	6.30	6.23	6.19	6.31
LSD	0.188	0.153	0.230	0.261	0.239	0.223	0.131	0.110	0.264
Significance	NS	NS	NS	NS	NS	NS	*	**	*

5.3.3 Dietary digestibility

The results of the digestibility measurements made during the fourth and fifth weeks of each experimental period are presented in Tables 5.6 - 5.9. The only parameter for which the difference between silages was significant ($P < 0.05$) was DCP content. In this case the higher level of total N in Silage D, combined with

the higher coefficient of digestibility, resulted in a DCP value some 27% greater than that of Silage C.

Table 5.6 Amount of dry matter consumed during digestibility trials

	Dry matter intake		
	($g\ d^{-1}$)	($g\ kg^{-1}$ LW d^{-1})	($g\ kg^{-0.75}$ LW d^{-1})
Silage C	772	13.4	36.7
Silage D	847	14.3	39.6
Dried grass	997	17.3	47.5
LSD	351.9	7.89	20.3
Significance	NS	NS	NS

Table 5.7 Digestibility coefficients

	Dry matter	Gross energy	Organic matter	Total N
Silage C	0.703	0.700	0.725	0.627
Silage D	0.699	0.702	0.720	0.660
Dried grass	0.661	0.651	0.688	0.693
LSD	0.0550	0.0579	0.0544	0.0486
Significance	NS	NS	NS	NS

Table 5.8 Concentration of digestible and metabolisable nutrients in the diets

	DOMD ($g\ kg^{-1}$ DM)	DE ($MJ\ kg^{-1}$ DM)	ME ($MJ\ kg^{-1}$ DM)	DCP ($g\ kg^{-1}$ DM)
Silage C	671	13.0	10.8	55.1
Silage D	665	13.8	11.3	70.1
Dried grass	642	12.2	9.8	123.2
LSD	50.5	1.08	0.98	6.71
Significance	NS	NS	NS	**

Table 5.9 Retention of N and the excretion of N and energy in the urine

	N retention ($g\ d^{-1}$)	Urinary excretion (% of intake)	
		N	Gross energy
Silage C	-0.01	72.1	4.21
Silage D	1.38	59.2	4.60
Dried grass	6.02	48.1	5.35
LSD	2.667	31.70	0.762
Significance	NS	NS	NS

5.3.4 Rate of flow of digesta

Mean daily intakes of dry matter, recorded during the period when measurements of flow rate were being made, are given in Table 5.10. For the two silages, these intakes were slightly higher than had been recorded in the preceding phase during which digestibility was measured (compare Table 5.6).

In Table 5.11 the reconstitution factors and the apparent recoveries of Ru and Cr administered in the flow rate marker, are presented. In one of the few instances in which a significant difference was noted between the silages in this section of Experiment Two, the magnitude of the reconstitution factor was greater ($P < 0.05$) for Silage C than for Silage D.

The measured, or derived, estimates of the rate of flow of a range of nutrients in abomasal digesta are presented in Table 5.12, while in Table 5.13 some of these rates of flow are expressed as proportions of nutrient intake.

In Table 5.14, the three relative terms which express the extent to which dietary N has been degraded and incorporated into microbial N are presented. The calculation of the corrected degradability term uses a value of 1.5 g d^{-1} as an estimate of the flow of endogenous N at the abomasum (Smith et al, 1976).

The extent of ruminal degradation of digestible organic matter and the concentration of DOMR in the diets, are shown in Table 5.15. The apparent efficiency of synthesis of microbial N, expressed in two ways, is presented in the same table.

Finally, in Table 5.16, the balance between the supply of nitrogenous nutrients and those providing energy, is given in three different ways. The last term, g degradable N kg^{-1} DOMR, describes

the balance between the provision of nitrogen and energy for microbial growth in the rumen.

Table 5.10 Amount of dry matter consumed during measurement of flow rate

	Dry matter intake		
	($g\ d^{-1}$)	($g\ kg^{-1}$ LW d^{-1})	($g\ kg^{-0.75}$ LW d^{-1})
Silage C	799	13.6	37.6
Silage D	883	15.8	43.0
Dried grass	1000	17.4	47.7
LSD	361.4	7.6	20.00
Significance	NS	NS	NS

Table 5.11 Reconstitution factor 'R' and the apparent recovery of administered markers

	Reconstitution factor 'R'	Apparent recovery (%)	
		Ru	Cr
Silage C	-0.294	97.7	98.1
Silage D	-0.087	92.6	100.8
Dried grass	-0.168	93.9	97.6
LSD	0.1618	14.67	21.01
Signficance	*	NS	NS

Table 5.12 Flow of nutrients at the abomasum

	Nutrient flow ($g\ d^{-1}$)				
	Dry matter	Organic matter	Total N	Non-ammonia N	Microbial N
Silage C	636	452	19.2	15.2	7.2
Silage D	567	415	18.3	15.3	7.3
Dried grass	625	514	27.3	25.7	10.2
LSD	413.1	307.8	11.47	11.02	4.45
Significance	NS	NS	NS	NS	NS

Table 5.13 Flow of nutrients at the abomasum, related to nutrient intake

	Nutrient flow				
	Organic matter ($g\ kg^{-1}$ OM intake)	Total N ($g\ kg^{-1}$ total N intake)	Non-ammonia N ($g\ kg^{-1}$ total N intake)	Non-ammonia N ($g\ kg^{-1}$ non-ammonia N intake)	Microbial N ($g\ kg^{-1}$ total N intake)
Silage C	581	1439	1155	1243	634
Silage D	522	1249	1061	1142	506
Dried grass	551	959	904	913	357
LSD	249.2	656.8	634.2	675.0	156.2
Significance	NS	NS	NS	NS	NS

Table 5.14 Microbial N content of abomasal digesta and the apparent degradability of dietary N in the rumen. The degradability terms are obtained using the equation in the Agricultural Research Council's review (ARC, 1980)

	Microbial N content in abomasal digesta (g kg^{-1} total N)	Uncorrected degradability of dietary N	Corrected degradability of dietary N
Silage C	415	0.182	0.350
Silage D	398	0.295	0.370
Dried grass	396	0.398	0.450
LSD	178.8	0.5672	0.6273
Significance	NS	NS	NS

Table 5.15 Dietary content of DOMR, and the apparent efficiency of synthesis of microbial N

	Proportion of DOM apparently digested in the rumen	Dietary content of DOMR (g kg^{-1} DM)	Efficiency of synthesis of microbial N	
			(g N kg^{-1} DOMR)	(g N MJ^{-1} ME intake)
Silage C	0.594	404	25.9	0.864
Silage D	0.685	464	18.0	0.671
Dried grass	0.640	411	24.8	1.044
LSD	0.238	192.4	20.67	0.257
Significance	NS	NS	NS	NS

Table 5.16 Concentrations of non-ammonia N in the DOM and DOMR, and of degradable N in the DOMR

	Non-ammonia N content		Degradable N content
	(g kg^{-1} DOM)	(g kg^{-1} DOMR)	(g kg^{-1} DOMR)
Silage C	20.4	39.9	10.8
Silage D	23.6	47.7	15.1
Dried grass	44.0	93.2	30.9
LSD	3.81	18.06	27.90
Significance	NS	NS	NS

5.4 DISCUSSION

5.4.1 Composition of diets

The important differences in composition between the two silages, shown in Table 5.2, may be conveniently divided into those which appear to be the result of the imposed pre-ensiling treatment and those caused by variability in the ensiled crop.

As can be seen from Table 5.1, the content of total N in the grasses differed by 3.8 g kg^{-1} DM, or 29% of the N content of the grass for Silage C. A similar difference (3.0 g N kg^{-1} DM, 21% of the N content of Silage C) was observed in the silages (Table 5.2) and it therefore seems unlikely that the pre-ensiling application of formic acid was responsible for this. In an experiment designed primarily to investigate the effects of a particular treatment, it is unfortunate that such a pronounced difference, most likely attributable to variation within the field in the level of total N in the grass, should have occurred. The problem may have been exacerbated by the difficulty of identifying the exact location within the larger silo occupied by the material corresponding to that placed in the smaller bunker silo. The difference between the silages would have been smaller had both been made in small silos, and the grass for each collected from alternate swaths in the field.

Other workers have encountered similar problems, and have reported treated and untreated silages which differed in their content of total N, although the applied treatment was not expected to influence the parameter. Gill et al (1979) made a grass silage, treated with formic acid and formalin, which had a total N content 1.8 g kg^{-1} DM less than that of the untreated control. Donaldson and Edwards (1980) ensiled unwilted ryegrass, either untreated

or treated with one of three additives containing formaldehyde. All the treated silages had higher contents of total N than the control, the differences ranging from 0.8 g kg^{-1} DM (2.6% of the N content of the control) to 4.5 g kg^{-1} DM (15% of the N content of the control). Similar observations have been made when comparing wilted and unwilted silages, and Morgan et al (1980b) found that the total N content of their wilted material was 2.4 g kg^{-1} DM less than that in an unwilted control. In this case the difference had also been observed in the grass which was ensiled.

Care must be taken when asserting that such differences are not due to the additive treatment, although work with silages made on a test tube scale suggests that the assumption is safe. McDonald et al (1983) demonstrated no significant difference between the levels of total N in silages treated with 4 or 8 l formic acid t^{-1}, and an untreated control. However, as Henderson, McDonald and Anderson (1982) illustrated, care must be taken when extrapolating from the results of test tube experiments to a larger scale, when loss of effluent containing varying amounts of nutrients can occur.

McDonald and Edwards (1976) stated that at the commercially recommended rate of 2.3 l t^{-1}, formic acid only partially restricted the natural fermentation of grass silage. More acid was applied in the present experiment (3.4 l t^{-1}), but the suppressive effects on the fermentation process appear to have been slight. The concentration of acid and alcohol products of microbial fermentation[1]. was slightly lower for Silage D than for Silage C (165 vs 183 g kg^{-1} DM) and some water-soluble carbohydrate, albeit a small proportion of that which was originally present, remained in the

1. Ethanol + lactic acid + acetic acid + propionic acid + n-butyric acid

treated silage. The higher ethanol content of Silage D may be attributed to the differential sensitivity of yeasts, which are responsible for the production of most of this alcohol in silage, and bacteria to formic acid (McDonald and Whittenbury, 1973). McDonald et al (1983) found that silage made from grass treated with formic acid at the rate of 4 l t^{-1} contained significantly ($P < 0.001$) less fermentation acids (17.5 vs 90.3 g kg^{-1} DM), but significantly ($P < 0.05$) more ethanol (26.4 vs 19.8 g kg^{-1} DM), than an untreated control. At a higher application rate of 8 l t^{-1}, formic acid inhibited the activities of both groups of micro-organisms, as evidenced by fermentation acid and ethanol levels of 4.0 and 1.0 g kg^{-1} DM respectively.

The effect of formic acid in suppressing bacterial fermentation is often manifested as a reduction in the extent of proteolysis (Barry et al, 1978a; McDonald et al, 1983). This was observed in the current experiment, with the protein content (g kg^{-1} total N) of Silage D being 25% greater than that of Silage C. The magnitude of this effect is much less than was reported by McDonald et al (1983) when formic acid at 4 l t^{-1} produced a silage with a protein N content 82% greater than that of an untreated control.

In the present study, no specific anti-clostridial effects could be demonstrated for formic acid, perhaps because the fermentation quality of Silage C was, in any case, very good. Barry, Mundell, Wilkins and Beever (1978b) demonstrated that formic acid, applied to precision-chopped grass at the rate of 1.5, 3.0 or 6.0 l t^{-1}, did reduce the formation of the amino acids, alanine α-aminobutyrate and δ-aminobutyrate, associated with clostridial activity.

5.4.2 Ruminal metabolism

On first inspection, little evidence can be found in the data in Table 5.4 - shown diagrammatically in Fig 5.2 - to support the contention that inefficient utilisation of silage N results from excessive production of ammonia in the rumen (McDonald and Edwards, 1976; Morgan et al, 1980a; Donaldson and Edwards, 1980). The peak concentration of ammonia N observed when feeding dried grass (344 mg l^{-1}) was significantly ($P < 0.01$) higher than that for Silage C (169 mg l^{-1}) or Silage D (191 mg l^{-1}). Much higher peak levels of ammonia N have been reported elsewhere when grass silages were fed to sheep. Donaldson and Edwards (1980) recorded a peak concentration of 470 mg l^{-1} for ammonia N in the rumen of sheep fed twice daily and Beever et al (1977) observed a concentration of 260 mg ammonia N l^{-1}, 3.5 h after offering untreated ryegrass silage to wethers. In contrast Morgan et al (1980a) observed a peak ruminal ammonia N content of only about 145 mg l^{-1} when a grass silage of low total N content was fed to sheep.

In the present experiment, the concentration of ammonia in the rumen immediately before feeding was much higher for the dried grass than for either of the silages. The magnitude of the peak 'generated' from each baseline value at 09.00 h was therefore less than the maximum observed concentration, and the reduction was greatest with the dried grass. The baseline-to-peak changes in ruminal ammonia N content were +34 and +75 mg l^{-1} for Silage C and Silage D respectively. These compare with values of +295 mg ammonia N l^{-1} and +60 mg l^{-1} observed by Donaldson and Edwards (1980) and Morgan et al (1980a) respectively.

Morgan et al(1980b) related peak rumen ammonia level to the amount of N consumed in the food and found that this mechanism could entirely explain a high ammonia N peak observed for one of their diets. A similar computation with the 'baseline-to-peak' increases in concentration recorded in the present experiment gives interesting comparative figures, using units of 'mg ammonia N g^{-1} N intake' (mg g^{-1}N). These are 3.1, 5.2 and 3.8 mg g^{-1} N for Silage C, Silage D and the dried grass respectively. The silages may now be viewed as being at least as 'efficient' as the dried grass in promoting high ruminal ammonia levels, despite the fact that both have a lower content of total N and give lower ammonia N concentrations in absolute terms. The specific production of ammonia appears to be higher from Silage D than from Silage C although no ready explanation for this can be found in their compositions (Table 5.2). It can be seen that the proportion of total N present as protein N was, in fact, slightly higher for the formic acid-treated material. When the results of other workers are expressed in the same manner, differences between species of ruminant are apparent. Thus Morgan et al (1980b) observed values of 0.42 and 0.73 mg ammonia N g^{-1} N intake respectively when feeding fresh and wilted silages to steers. When feeding untreated ryegrass silage to sheep, the same workers (Morgan et al, 1980a) found that the value was 5.3 mg g^{-1} N, while Donaldson and Edwards (1980) observed a value of 12.6 mg g^{-1} N in their experiment in which the access of sheep to the food was restricted. Siddons and Paradine (1983) noted that dried grass or dried grass and barley mixtures induced higher concentrations of ammonia in the rumen liquor of sheep than in that of steers, when the diets were fed to both species at a

maintenance level of energy intake. There were significant differences between diets, and these dietary responses were similar in both species.

The high figure observed by Donaldson and Edwards (1980) illustrates the influence which the rate of intake of dry matter has on ruminal ammonia levels. In their study, very high rates of dry matter intake were achieved by restricting access to the silage to 2 h at each meal (09.00 h - 11.00 h and 21.00 h - 23.00 h). In addition, the silage was highly digestible and of high total N content, having been made from grass cut before ear emergence. Although the rate was not constant throughout the day, silage was consumed over a 12 h - 24 h period in the present study, whereas the grass pellets were generally eaten within 1 - 2 h. Michalowski (1979) found that offering sheep their daily ration in two meals instead of one resulted in higher ruminal ammonia N concentrations, probably because rates of dry matter intake were higher.

The diurnal trough in the ammonia concentration noted for dried grass (see Fig 5.2) was, in fact, larger (baseline-to-trough = 146 mg ammonia N l^{-1}) than the peak which resulted when the food was consumed. The effect is possibly so pronounced because of the very episodic manner in which the dried grass was consumed over the 24 h period. It is likely that the balance, between production resulting from the deamination of dietary and endogenous N components, and utilisation as ammonia is incorporated into bacterial protein, influences the ruminal ammonia concentration which is observed. This has certainly been noted in simple <u>in vitro</u> systems in which, because of bacterial incorporation, ammonia concentrations have remained stable while degradation of food N continued (Siddons

et al, 1982). Thus the diurnal pattern shown by dried grass in Fig 5.2 may be explained in terms of a rapid initial rise resulting from degradation of dietary nitrogenous compounds in the first hour, followed by an equally precipitious fall as bacterial synthesis gathered pace but no more dietary N entered the rumen. The subsequent rise to the intermediate pre-feeding level may reflect a balance between degradation of recycled nitrogenous compounds and bacterial synthesis. The peak and trough pattern is not nearly so pronounced with the silages where substrate was entering the rumen over a much more protracted period. Neither Donaldson and Edwards (1980) nor Morgan et al (1980a) observed diurnal troughs in ruminal ammonia concentrations when silages were offered to sheep twice daily. However when silage was supplemented with a large quantity of soyabean meal such that the pre-feeding baseline and post-feeding peak were both elevated, Morgan et al (1980a) recorded a pattern, of ammonia concentration against time, very similar to that shown for dried grass in Fig 5.2.

The slight increase in ruminal ammonia concentration in the period immediately before feeding (compare 07.00 h and 09.00 h in Table 5.4 and Fig 5.2) has been noted by other workers (Wohlt, Clark and Blaisdell, 1976). With the silages, this may be the result of increased dry matter intake in response to increased activity in the animal house and the impending arrival of fresh food. This mechanism cannot apply to the dried grass, since invariably none was left at 07.00 h.

Whether the ruminal ammonia concentrations illustrated in Fig 5.2 are adequate or excessive, depends entirely on how adequacy is assessed (see Table 2.4.1, Section 2.4.4). In general, the

levels observed in the present experiment were adequate to support maximal synthesis of microbial protein or maximal flow of non-ammonia N to the small intestine (see Table 2.4.1; Pisulewski et al, 1981; Buttery and Lewis, 1982). However, other than in the case of dried grass in the period immediately after feeding, the levels were not as high as those reportedly required to maximise the rate of degradation of dry matter in the rumen (Mehrez et al, 1977; Wallace, 1979; Buttery and Lewis, 1982).

Ruminal pH (Table 5.5, Fig 5.3) reflects the rate of intake of dietary carbohydrate and its degradation to volatile fatty acids. It is not surprising therefore to find that the rate of fall and the magnitude of the reduction are greater for the dried grass. This was consumed more rapidly and contained smaller particles more accesible to degradation by rumen microbes. When the different patterns of intake of the three diets are considered, it seems unlikely that there is any great difference in the specific production of volatile fatty acids from dietary carbohydrate.

With regard to wasteful loss of ammonia from the rumen, it is important to bear in mind the findings of Chalmers et al (1971) that the main route for ammonia loss from the rumen is through concentration-dependent diffusion of NH_3. The concentration of NH_3 is determined by total concentration of ammonia and pH; the higher the pH, the higher is the concentration of NH_3 (and the lower the concentration of NH^+_4). The higher rumen pH observed with the silages in the current experiment must have resulted in higher NH_3 concentrations, but these differences would not have been significant since all pH's were low compared with the level of 7.3 which Chalmers et al (1971) quoted as being necessary to

induce large losses of ammonia from the rumen.

In Fig 5.4, the non-glucogenic ratio (NGR), has been plotted against time for each diet. For all three diets, the NGR drops immediately in response to feeding. This initial drop is followed by a rise which, in the case of Silage D and the dried grass, peaks some 8 to 12 h after feeding, at a value considerably greater than the pre-feeding level. For Silage C, the fall in the first hour after feeding is much larger and the following 23 h are spent regaining the initial level. All the diets have NGR's which are higher than the upper limit of the range of 2.25 - 3.0 identified by Ørskov (1975) as that within which the efficiency of utilisation of ME for fattening is maximised. Production of milk remains highly efficient over the wider range of 3.0 - 4.0 (Ørskov, 1975) and the NGR for the untreated Silage C remains, for the most part, in this zone. It appears from Fig 5.4 that the formic acid additive has adversely affected the efficiency with which the energy of the silage would be used for productive processes. Donaldson and Edwards (1980) observed similar patterns for diurnal variation in the NGR when silages were fed to sheep. They also found that a silage treated with a formic acid/formalin mixture produced a higher NGR than an untreated control and concluded that this could have a detrimental effect on the efficiency of utilisation of ME for fattening in particular.

5.4.3 Dietary digestibility

Perhaps the most striking feature of Tables 5.6 - 5.9 is the lack of significant differences between the silages. In contrast with the later measurements of flow rate (see Section 5.4.4), this cannot be attributed to a large amount of random variation in the

measurements. The coefficients of variation for the digestibilities and digestible and metabolisable nutrient contents shown in Tables 5.7 and 5.8 were all below 10%.

As in Experiment One, the intention in this experiment was to achieve uniform intakes of about 1 kg DM for each diet. This was not satisfactorily achieved for the silages, although the large LSD ensured that neither intake could be shown to differ significantly from 1000 g d^{-1}. The recorded intakes of 772 g d^{-1} and 847 g d^{-1} for Silages C and D, respectively, were lower than the predicted appetite of 990 g d^{-1} for growing sheep of the same average weight (ARC, 1980). The silages were therefore effectively being fed ad libitum, and the apparent trend for the intake of Silage D to be higher than that of Silage C is noteworthy. Donaldson and Edwards (1976), Hinks and Henderson (1977) and Barry et al (1978a) have all cited or observed increased voluntary intake of dry matter as a beneficial effect of formic acid as a pre-ensiling treatment. When such effects have been observed, they have usually been attributed to the restriction of the formation of fermentation products (McDonald and Edwards, 1976; Barry et al, 1978a). In view of their compositions, it is therefore unsurprising that marked differences were not observed between the intakes of Silage C and Silage D. Hinks and Henderson (1977) noted that pre-ensiling treatment with formic acid increased the intake of dry matter when the silage was fed to a penned group of 40 Friesian steers, but observed no similar effect when the silage was fed to caged animals in a metabolism trial.

Formic acid did not significantly influence the digestibility of any of the parameters recorded (dry matter, organic matter,

gross energy and total N) nor the contents of digestible organic matter or digestible and metabolisable energy. In the case of digestible crude protein, the combination of a higher digestibility with the higher level of total N in Silage D, resulted in a higher ($P < 0.01$) value for the treated silage. This observation may, in part, be artefactual since the higher apparent digestibility of Silage D could simply be the result of its higher content of total N. With a relatively constant endogenous, or metabolic, component in faecal N, a food of higher total N content will have a higher apparent digestibility of that N than a similar food with the same true N digestibility, but a lower total N concentration (McDonald et al, 1973).

The excretion of N in the urine, recorded for the silage diets, is high (Table 5.9) and yet, as in Experiment One, the much higher figure recorded for retention of N with the dried grass means that the high N excretion with the silage diets cannot be explained in terms of the provision of a nutrient for which the animal has no use.

5.4.4 Rate of flow of digesta

The most prominent feature of Tables 5.10 - 5.16, which contain the results from the measurements of digesta flow rate, is the lack of statistically significant effects attributable to differences between the silages. This can occur even when the error term, and hence the random variability of the experiment, is small, if the observed mean values are similar. However, closer examination of Tables 5.10 - 5.16 reveals that the LSD's are all large in comparison with the mean values to which they apply. In this respect they are notably different from the LSD's in Experiments One, Three

and Four (see Sections 4.3.3, 6.3.4, 7.3.4). In each of the current series of experiments, the random variability, which can be expressed in the coefficient of variation (CV), associated with the measurements of flow rate was higher than that encountered when more straightforward determinations of dietary digestibility and ruminal metabolism were made. In the present experiment, the CV% values for digestibility measurements were all around 10%. Although this is perhaps higher than desirable, and slightly higher than was obtained in the other experiments, it remains acceptable for animal experimentation. In the case of the measurements reported in Tables 5.10 - 5.16, however, values from 17% (apparent recovery of Ru) to 168% (uncorrected degradability of dietary N) were calculated for the CV. Since there were no important differences in procedure or in analytical techniques between this experiment and the others in the series, it is difficult to explain why the random variation is so high in this case.

The effect of the excessive random variability, which renders the estimates of all the mean values in this part of the experiment imprecise, is to preclude unequivocal comment on the differences between the silages and the effects of formic acid as a pre-ensiling additive. Since the 95% confidence interval (mean value $\pm$ LSD) for each value is so large, there is little to be gained from noting that the values lie within the range that has been previously reported for the parameter. Nevertheless, some comparisons can be drawn between the present results and those which have been reported previously.

As in Experiment One, no material differences in dry matter intake developed between the digestibility trial and the

measurement of flow rate (compare Tables 5.4 and 5.10). The recoveries of Ru and Cr (Table 5.11) were more satisfactory in the present study. The mean values are very close to those reported by Macrae and Evans (1973) for Ru and by Beever et al (1978) for Ru and Cr.

The magnitude of the reconstitution factor, R, was greater for Silage C than for Silage D ($P < 0.05$). In both cases, the sign of R was negative indicating that centrifuged digesta had to be removed from the sampled digesta to reconstitute true digesta. The 95% confidence limits of the R value for Silage D encompass 0, indicating that no real bias existed in the sample of digesta which was obtained. For Silage C, however, the sampled material clearly contained solids in greater proportion than did the digesta flowing past the cannula. This sampling bias was noted in Experiment One and was reported by Faichney (1975b) and Beever et al (1978).

The rates of flow of the various nutrients, Tables 5.12 and 5.13, again exhibit high CV's ranging from 34 to 73%. The higher content of total N in Silage D, combined with an apparently higher intake of that silage, did not alter the passage of total N or non-ammonia N to the abomasum. Flow of total N (g kg^{-1} N intake) was again negatively correlated with the concentration of N in the diet although this trend could not be translated into significant differences between any of the diets. Barry et al (1978a) found that the retention of N was higher when silage treated with formic acid was fed to sheep in place of an untreated control and that this was partly explained by superior N supply per unit N intake from the treated material.

For the values of degradability in Table 5.14, random variability becomes totally unacceptable with CV values of 168% and 139% for uncorrected and corrected degradabilities, respectively. These compare with values of 42% and 32% in Experiment One, which were themselves considered to be unsatisfactory. Although formic acid may have appreciable effects on the composition of the nitrogenous fraction of silage, its effects on the subsequent digestion and metabolism of that nitrogen appear to be less important. Siddons et al (1982), using in vitro and in sacco procedures, found that formic acid had relatively minor effects on the solubility of silage N, when compared with additives containing formaldehyde. Similarly, McDonald et al (1983) observed that, although pre-ensiling treatment with formic acid reduced the rate of loss of total N from silages incubated in Dacron bags in the rumen of cattle, after a period of 24 h, only silages treated with formalin had lost less N than had the untreated control.

The efficiency of synthesis of microbial N (g kg^{-1} DOMR) has a high degree of random variability, shows no difference between diets and, with due regard to the imprecision of the estimates, agrees with previously reported values (ARC, 1980; Thomas et al, 1980; Thomson, Beever, Lonsdale, Haines, Cammell and Austin, 1981). As in Experiment One, when expressed as 'g microbial N MJ^{-1} ME intake', the values observed in the present experiment are low. Their 95% confidence intervals do not encompass the mean value proposed for all diets by the Agricultural Research Council (ARC, 1984). They do, however, agree reasonably well with the single value of 0.71 g N for grass silage eaten by sheep, which was included in the Council's data (ARC, 1984).

5.5 CONCLUSIONS

It is evident from the present study that the elimination of the effects of all variables other than those being specifically studied, is as important when experimenting with silages as with any other biological material. In particular, when assessing the effects of a pre-ensiling additive, it is desirable that the grass used for the untreated and treated silages be as similar as possible in composition, and be ensiled in separate similar silos. Although probably not demonstrated in this experiment, the observations by other workers that the scale on which ensiling is carried out can affect both natural fermentation and the efficacy of additives (Meiske, Linn and Goodrich, 1975), reinforces these recommendations. With due consideration to the limitations imposed by different starting materials, it seems reasonable to conclude from the present work that formic acid can influence the composition of grass silage. The effects, which include reduced proteolysis and increased production of ethanol, will, however, be slight when the untreated material is itself well preserved.

Measurements of ruminal ammonia levels in this study indicate that a mechanism more complex than the rapid loss of ammonia, present at high concentration, must be postulated if an explanation for poor efficiency of utilisation of silage N is required. There are indications, however, that specific production of ammonia (i e per unit N intake) may be higher for silages than for other forages, and that ammonia levels may be more directly influenced by the rate of intake of nitrogenous substrate for microbial fermentation.

In contrast with many other measurements, the effect of formic acid on the pattern of carbohydrate fermentation in the rumen was

substantial. The treated silage had a higher NGR which could result in less efficient utilisation of dietary ME. Such measurements are not often reported, but those which have, support the current findings on the possible deleterious effects of formic acid (Donaldson and Edwards, 1980).

Attempts to measure the rate of flow of nitrogenous nutrients to the abomasum served only to illustrate the problems which can arise when the level of random variability in an experiment is high. It was instructive to note that two of the parameters conventionally used to assess the performance of such experiments, recovery of the administered markers and the magnitude of the reconstitution factor, were, superficially, quite acceptable. Since no particular reason could be found for the lack of precision, few conclusions can be drawn other than that the possibilities for such failure exist and should be borne in mind before choosing the techniques for a particular study.

6. EXPERIMENT THREE

6.1 INTRODUCTION

Wilting, the technique of increasing the dry matter content of ensiled herbage by a period of field drying, has been discussed in Section 2.1.3.6. It is a popular feature of silage making in the United Kingdom, being advocated by the Government advisory services (MAFF, 1977). Its importance may be gauged from a recent survey of the quality of silages made in the Spring and Summer of 1985 in the English Midland counties of Herefordshire, Worcestershire and Warwickshire (ADAS, 1985). Despite unfavourable weather conditions, with rainfall in the region being 50% more than the long term average, the mean dry matter content of the silages was 276 g kg^{-1}.

Although the effects of wilting on the processes of silage making are well understood, their influence on the composition and, in particular, the feeding value of silage are more contentious. The European Grassland Federation decided, in 1980, to coordinate a series of experiments in several European countries, designed to investigate the effects of wilting on losses during the ensilage process, and on the feeding value of silage (Zimmer and Wilkins, 1984). Part of the contribution of the Edinburgh School of Agriculture was an unwilted silage treated with formic acid (Silage D from Experiment Two) and an untreated wilted silage (Silage E in the present experiment, see Section 6.2 below). The opportunity was therefore taken to complement these silages with unwilted, untreated, and wilted, formic acid-treated, materials. The unwilted silages were investigated in Experiment Two and their wilted analogues were tested in the present study. Comparisons within Experiment Three allow elucidation of the

effects of formic acid on wilted silage. Comparisons between the second and third experiments demonstrate directly the effect of wilting, and also any interaction between the effect of formic acid and the dry matter of the herbage ensiled.

6.2 METHODS AND MATERIALS

6.2.1 Experimental design and procedure

The animal studies of Experiment Three were conducted between 11 November 1982 and 5 March 1983. The three experimental periods were of 5 weeks' duration and followed the format described in Section 3.2 above.

Eight sheep (numbers 32, 37, 91, 155, 487, 510, 716 and 1280) were used in this experiment. The average weight of these animals over the course of the experiment was 54.3 kg, with minima and maxima of 40.4 and 72.0 kg. The allocation of diets for each animal is shown in Figure 6.1. The schedule illustrated in Fig 6.1 was subject to the following disruptions.

(i) No sample of abomasal digesta could be obtained from Sheep 32 in Period 1. As a result, no values were determined for any of the parameters of digesta flow rate for dried grass in Column III. The condition of the sheep showed no signs of improving when the animals were introduced to their diets for Period 2, and Sheep 32 was therefore replaced by Sheep 487 for Periods 2 and 3.

(ii) Towards the end of the second period, the area around the abomasal cannula of Sheep 716 began to show symptoms of impending 'rejection' of the polythene fitment. The sheep's flank, in the region of the cannula, became swollen and inflamed, exuded pus from ruptures in the skin surface and

caused discomfort to the animal. The sheep was withdrawn from the trial at the end of the period and was replaced by Sheep 1280 for Period 3. Problems of this nature were, unfortunately, relatively common with the particular surgical preparations employed in the present series of experiments (see Section 8.2.1).

(iii) No sample of digesta could be obtained through the abomasal cannula of Sheep 510 in Period 2. Thus, no parameters of digesta flow rate were determined for Column II (Silage E). In this case the problem was only temporary, and the sheep was able to remain on trial, and performed satisfactorily, for the third period.

	Column					
	I	II	III	IV	V	VI
Sheep	716/1280	510	32/487	91	37	155
Period						
1	E	F	DG	E	F	DG
2	DG	E	F	F	DG	E
3	F	DG	E	DG	E	F

Figure 6.1 Allocation of three diets to six sheep over three experimental periods in Experiment Three. Untreated wilted silage (E), wilted silage treated with formic acid (F) and pelleted, dried grass (DG)

6.2.2 Diets

Three diets were offered in Experiment Three, two grass silages and the pelleted, dried grass which was fed in each experiment.

6.2.2.1 Silages

Silage E from this experiment, together with Silage D from Experiment Two, constituted part of the Edinburgh School of Agriculture's contribution to the Eurowilt programme of the European Grassland Federation (Adamson and Appleton, 1984; Zimmer and Wilkins, 1984). Silages C and F were additional treatments made specifically for the present studies. Silages E and F were therefore made from the same sward as that described in Section 5.2.2.1.

The crop was cut with a drum mower on 12 June 1981. Rain fell on 13 June and the grass was tedded the following day. This crop movement was repeated on the morning of 15 June, and in the afternoon the grass was windrowed. The swaths of wilted grass were lifted on 16 June using a precision-chop forage harvester. About 2.5 t of harvested material was ensiled in a concrete bunker silo 1.6 m wide, 2.8 m long and 1.6 m deep. As the grass was being placed in the silo, an 85% solution of formic acid (Add-F, BP Nutrition (UK) Ltd) was added at the rate of 3.0 l t^{-1}. The additive was applied periodically using a watering can and was spread evenly over the ensiled material after a thin layer (approximately 15 cm) of grass had been placed in the silo and consolidated. When the silo had been filled, the grass was covered with polythene sheeting which was sealed, and weighted with sandbags. The remainder of the grass from the field was ensiled in a walled clamp silo of approximately 50 t capacity. A record was kept of the approximate position, in this large silo, occupied by grass taken from the area in the field adjacent to that used to provide the grass ensiled in the bunker silo.

The clamp silo was opened on 3 February and the bunker silo on 4 March 1982, after ensiling periods of 232 and 261 d respectively. For each, surface spoilage was removed and approximately 0.7 t of silage (Silage E from the clamp silo and Silage F from the bunker silo) was placed on a clean concrete floor where it was thoroughly mixed. Representative samples were taken for subsequent analysis (see Section 3.4.1) and 240 polythene bags were each filled with 2.5 kg of silage. This provided slightly in excess of the required daily ration of 1 kg dry matter. The bags were sealed and then stored at -20°C until required. Silage was thus in refrigerated storage for between 252 and 393 d before being thawed and fed to the sheep.

6.2.2.2 Dried grass

The pelleted, dried grass was stored in a pest-proof container. At intervals during the course of the experiment, pellets were weighed into polythene bags. Sheep were offered a daily ration of 1.14 kg (1 kg dry matter).

6.3 RESULTS

6.3.1 Composition of diets

The composition of the grass which was ensiled is presented in Table 6.1. The compositions of the resulting silages are shown in Table 6.2. As in Experiment Two, steps were taken to minimise compositional differences between the silages, other than those imparted by the differential additive treatment. The dry matter contents were very similar, but the concentrations of total N differed by 3.2 g kg^{-1} DM with the value for Silage F being 18% greater than that for Silage E. The composition of the pelleted, dried grass is given in Table 6.3.

Table 6.1 Composition of standing crop and grass as ensiled

Component (g kg^{-1} DM unless otherwise stated)	Standing crop	Grass as ensiled
Dry matter (g kg^{-1})	196	432
Total N	13.9	15.7
Water-soluble carbohydrate	316	193
MAD fibre	268	326
GE (MJ kg^{-1} DM)	19.2	-

Table 6.2 Composition of silages

Component (g kg^{-1} DM unless otherwise stated)	Silage E	Silage F
Dry matter [1] (g kg^{-1})	404	393
Dry matter [2] (g kg^{-1})	438	416
pH (units)	4.33	4.31
Organic matter	922	922
Water-soluble carbohydrate	63.0	72.0
MAD fibre	351	322
Ethanol	2.9	3.0
Lactic acid	81.0	64.0
Acetic acid	23.0	19.0
Propionic acid	1.0	1.1
n-Butyric acid	1.0	1.0
Total N	17.6	20.8
Protein N	9.66	9.88
Protein N (g kg^{-1} total N)	550	476
Ammonia N	1.87	1.67
Ammonia N (g kg^{-1} total N)	107	80
Non-ammonia N	15.7	19.1
Non-ammonia N (g kg^{-1} total N)	893	920
Gross energy (MJ kg^{-1} DM)	18.4	18.4

1. Determined by drying to constant weight at 100°C

2. Determined by distillation with toluene (see Section 3.4)

Table 6.3 Composition of dried grass

Component (g kg^{-1} DM unless otherwise stated)	Pelleted, dried grass
Dry matter[1] (g kg^{-1})	877
Organic matter	933
Water-soluble carbohydrate	190
Total N	28.5
Protein N	23.4
Protein N (g kg^{-1} total N)	821
Ammonia N	0.3
Ammonia N (g kg^{-1} total N)	11.0
Non-ammonia N	28.2
Non-ammonia N (g kg^{-1} total N)	989
Gross energy (MJ kg^{-1} DM)	18.8

1. Determined by drying to constant weight at 100°C

6.3.2 Ruminal metabolism

Intakes of dry matter, recorded during the period when ruminal metabolism was being monitored, are given in Table 6.4. There were no differences of statistical or biological significance between the diets. Diurnal variation in the concentration of ammonia N in strained rumen liquor and of pH of rumen liquor, are shown in Tables 6.5 and 6.6 respectively. There were no significant differences ($P > 0.05$), between the silages, for either of these parameters at any of the sampling times. The contrast between the silages and the dried grass was again marked, and over most of the 24 h period the ammonia N and pH values produced by

feeding dried grass were different from those which resulted from the consumption of silage. The variation, with time, of ammonia N concentration, pH and the NGR of the volatile fatty acids in strained rumen liquor are shown in Figures 6.2, 6.3 and 6.4 respectively.

Table 6.4 Amount of dry matter consumed during measurement of ruminal metabolism

	Dry matter intake		
	g d^{-1}	g kg^{-1} LW d^{-1}	g $kg^{-0.75}$ LW d^{-1}
Silage E	925	17.2	46.4
Silage F	943	16.9	46.0
Dried grass	1000	18.5	52.3
LSD	157.2	3.11	8.42
Significance	NS	NS	NS

Table 6.5 Concentration of ammonia N in strained rumen liquor

	Ammonia N concentration (mg l^{-1})								
	07.00	09.00	10.00	11.00	12.00	13.00	15.00	17.00	21.00 h
Silage E	83	93	219	208	173	144	130	120	56
Silage F	77	119	207	238	188	169	127	108	82
Dried grass	186	199	340	341	298	243	148	100	78
LSD	37.4	45.8	57.3	34.4	42.5	49.1	44.6	56.0	46.5
Significance	NS	NS	NS	NS	NS	NS	NS	NS	NS

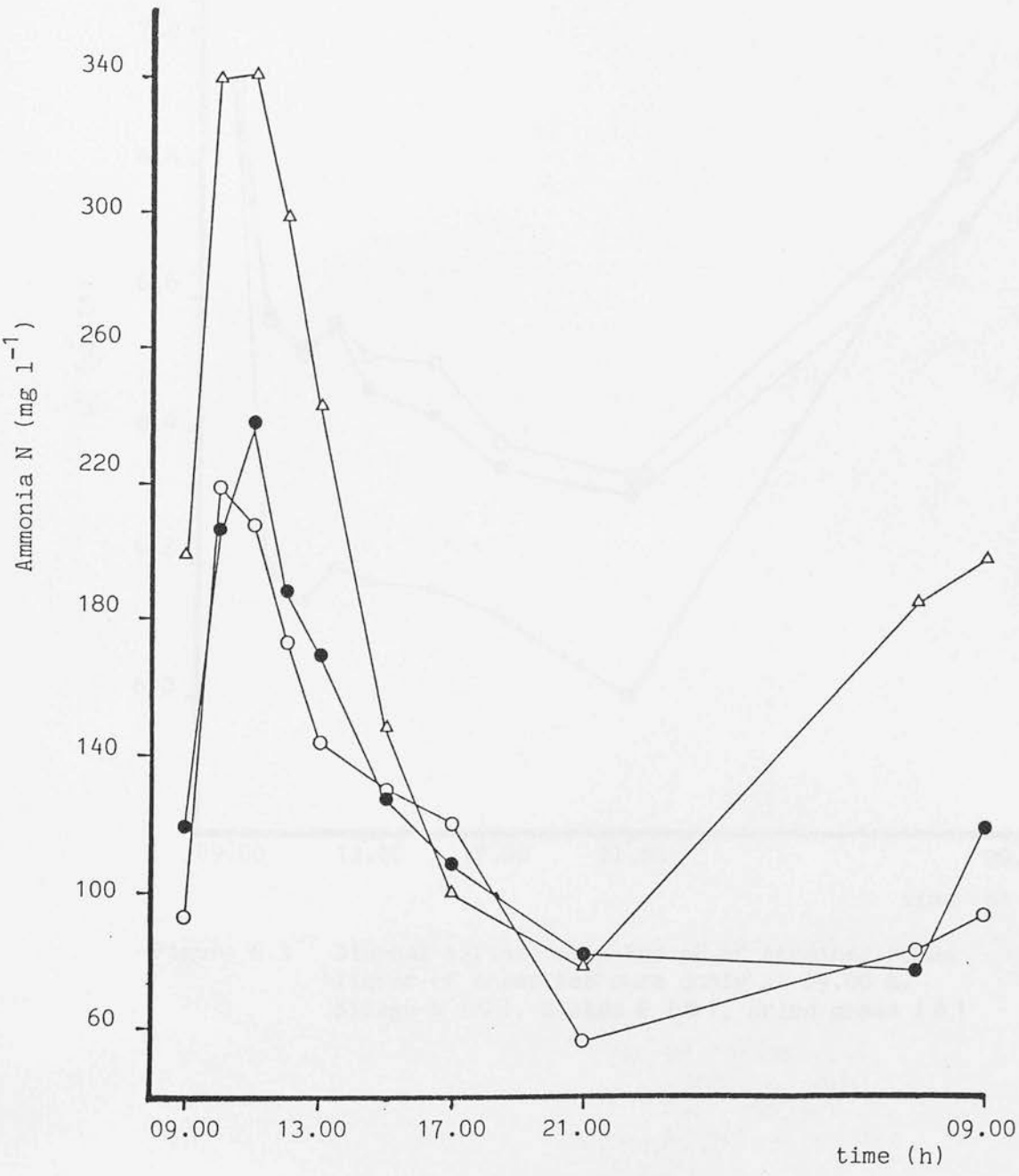

Figure 6.2 Diurnal variation in the concentration of ammonia N in strained rumen liquor of sheep fed once daily at 09.00 h. Silage E (○), Silage F (●), dried grass (△)

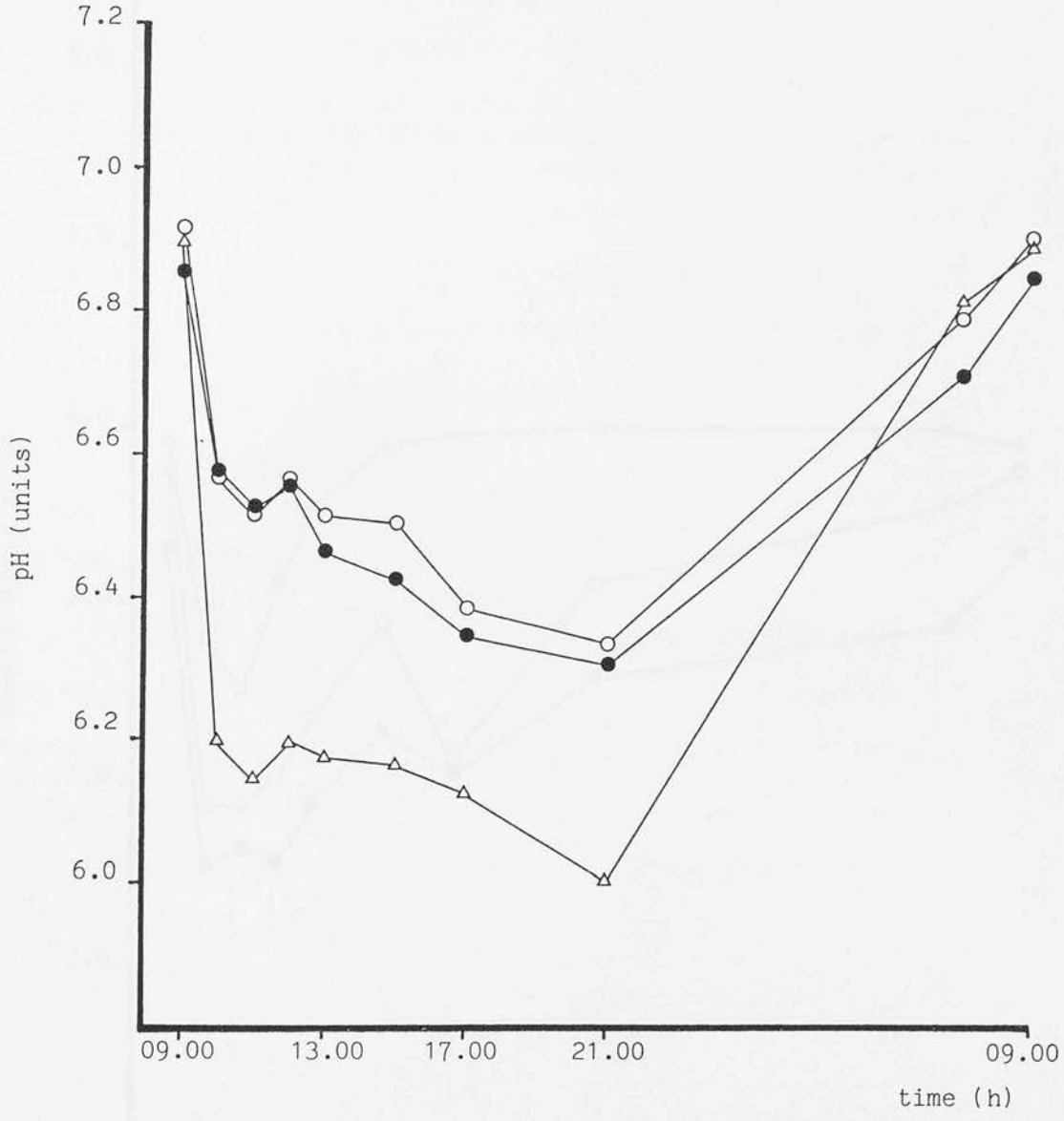

Figure 6.3 Diurnal variation in the pH of strained rumen liquor of sheep fed once daily at 09.00 h. Silage E (○), Silage F (●), dried grass (△)

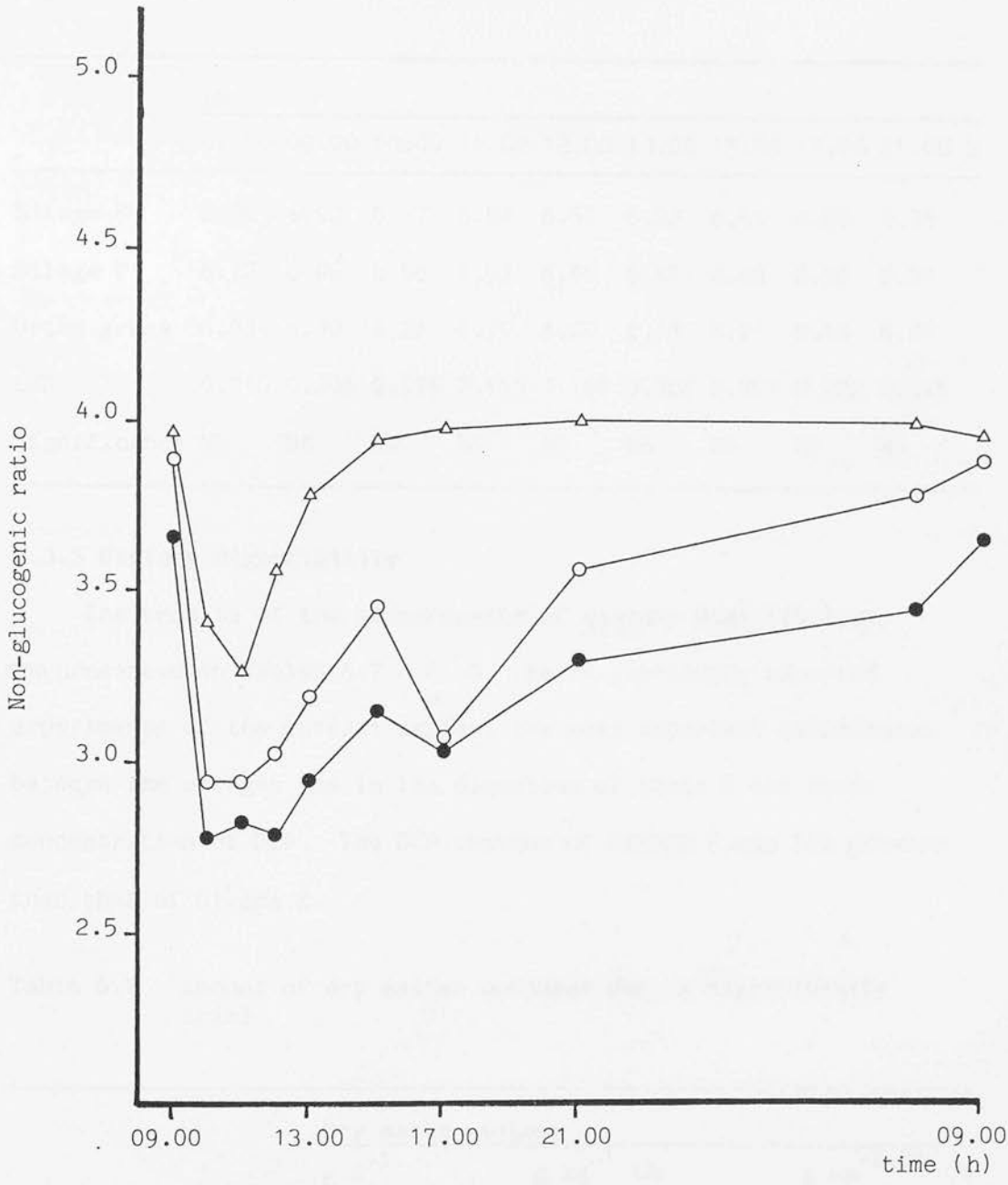

Figure 6.4 Diurnal variation in the non-glucogenic ratio of the volatile fatty acids in the strained rumen liquor of sheep fed once daily at 09.00 h. Silage E (○), Silage F (●), dried grass (△)

Table 6.6 pH of rumen liquor

	pH								
	07.00	09.00	10.00	11.00	12.00	13.00	15.00	17.00	21.00 h
Silage E	6.80	6.92	6.57	6.52	6.57	6.52	6.51	6.39	6.34
Silage F	6.72	6.86	6.58	6.53	6.56	6.47	6.43	6.35	6.31
Dried grass	6.83	6.90	6.20	6.15	6.20	6.18	6.71	6.13	6.01
LSD	0.290	0.206	0.175	0.155	0.147	0.208	0.251	0.222	0.341
Significance	NS	NS	NS	NS	NS	NS	NS	NS	NS

6.3.3 Dietary digestibility

The results of the measurements of dietary digestibility are presented in Tables 6.7 - 6.10. As in previously reported experiments of the current series, the most important differences between the silages was in the digestion of their N and their concentration of DCP. The DCP content of Silage F was 36% greater than that of Silage E.

Table 6.7 Amount of dry matter consumed during digestibility trial

	Dry matter intake		
	g d^{-1}	g kg^{-1} LW	g $kg^{-0.75}$ LW
Silage E	930	17.3	46.7
Silage F	898	15.9	43.4
Dried grass	982	18.0	48.9
LSD	144.9	2.91	7.65
Significance	NS	NS	NS

Table 6.8 Digestibility coefficients

	Dry matter	Gross energy	Organic matter	Total N
Silage E	0.692	0.671	0.704	0.624
Silage F	0.695	0.673	0.703	0.719
Dried grass	0.695	0.678	0.715	0.735
LSD	0.0419	0.0451	0.0425	0.0478
Significance	NS	NS	NS	**

Table 6.9 Concentration of digestible and metabolisable nutrients in the diets

	DOMD ($g\ kg^{-1}$ DM)	DE ($MJ\ kg^{-1}$ DM)	ME ($MJ\ kg^{-1}$ DM)	DCP ($g\ kg^{-1}$ DM)
Silage E	649	12.4	10.2	68.5
Silage F	648	12.4	10.3	93.3
Dried grass	667	12.6	10.4	131.2
LSD	39.5	0.84	0.74	6.90
Significance	NS	NS	NS	***

Table 6.10 Retention of N and the excretion of N and energy in the urine

	N retention ($g\ d^{-1}$)	Urinary excretion (% of intake)	
		N	GE
Silage E	3.52	40.7	3.60
Silage F	5.12	45.0	3.43
Dried grass	6.69	42.5	4.74
LSD	2.150	17.88	0.967
Significance	NS	NS	NS

6.3.4 Rate of flow of digesta

Amounts of dry matter consumed during the measurement of digesta flow are shown in Table 6.11. As with intakes recorded earlier in the sequence of metabolism measurements, there were no significant differences ($P > 0.05$) between the diets. However the figures for Silage F in Tables 6.4, 6.7 and 6.11 suggest a tendency for its intake to reduce gradually as each period progessed.

The reconstitution factors for sampled abomasal digesta, and the apparent recoveries of Ru and Cr in faeces are presented in Table 6.12. The milled, dried faeces samples from the faecal collections in Period 1 werediscarded before their contents of Ru and Cr had been determined. The analysis of variance techniques used for the other variates were not, therefore, appropriate for the remaining data, and the values shown in Table 6.12 are arthmetic means.

The rates of flow of a range of nutrients in abomasal digesta are presented in Table 6.13, and in Table 6.14 some of these rates

of flow are expressed as proportions of nutrient intake.

Three related terms which express the extent to which the nitrogenous fraction of the diet has been degraded in the rumen, are given in Table 6.15. The second, corrected, degradability term uses a value of 1.5 g d^{-1} as an estimate of the flow of endogenous N at the abomasum (Smith et al, 1976). The corrected degradability of the treated Silage F was greater than that of Silage E ($P < 0.05$).

The proportion of digestible organic matter which was apparently digested in the rumen and the concentration of that DOMR in the diets, are given in Table 6.16. In the same table the apparent efficiencies of synthesis ofmicrobial N, expressed per unit DOM digested in the rumen and per unit intake of ME, are presented.

Finally, Table 6.17 contains three terms which describe the balance between N and digestible or rumen degradable organic matter in the diets. The ratio of rumen degradable N to rumen degradable organic matter was higher for Silage F than for Silage E ($P < 0.05$).

Two relationships between parameters of the diets and passage of nitrogenous nutrients at the abomasum, are illustrated in Figures 6.5 and 6.6. In Fig 6.5, the relationship between intake of non-ammonia N and the passage of that nutrient at the abomasum, is shown. A significant positive relationship exists. In Fig 6.6 the flow of non-ammonia N (g kg^{-1} non-ammonia N intake) is plotted against the concentration of non-ammonia N in the diet (g kg^{-1} DOMR). In this case, no single straightline equation can be used to describe the whole data set. However, significant positive relationships do exist for individual diets.

Table 6.11 Amount of dry matter consumed during measurement of digesta flow rate

	Dry matter intake		
	g d^{-1}	g kg^{-1} LW d^{-1}	g $kg^{-0.75}$ LW d^{-1}
Silage E	911	17.8	48.1
Silage F	821	14.2	39.1
Dried grass	984	18.5	50.4
LSD	193.7	4.77	12.17
Significance	NS	NS	NS

Table 6.12 Reconstitution factor 'R' and recovery of administered Ru and Cr

	Reconstitution factor	Apparent recovery (%)[1.]	
		Ru	Cr
Silage E	-0.034	89.0	87.8
Silage F	-0.133	90.5	86.4
Dried grass	-0.165	94.0	90.5
LSD	0.3836		
Significance	NS		

1. Mean recoveries for Periods 2 and 3

Table 6.13 Flow of nutrients at the abomasum

	Nutrient flow ($g\ d^{-1}$)				
	Dry matter	Organic matter	Total N	Non-ammonia N	Microbial N
Silage E	645	503	21.4	19.9	11.0
Silage F	567	434	18.1	16.7	10.8
Dried grass	744	607	33.0	30.5	10.3
LSD	223.2	168.7	6.95	6.56	4.26
Significance	NS	NS	NS	NS	NS

Table 6.14 Flow of nutrients at the abomasum, related to nutrient intake

	Nutrient flow				
	Organic matter ($g\ kg^{-1}$ OM intake)	Total N ($g\ kg^{-1}$ TN intake)	Non-ammonia N ($g\ kg^{-1}$ TN intake)	Non-ammonia N ($g\ kg^{-1}$ NAN intake)	Microbial N ($g\ kg^{-1}$ TN intake)
Silage E	564	1267	1186	1325	650
Silage F	569	1087	1005	1093	618
Dried grass	665	1182	1093	1129	367
LSD	133.1	326.1	308.9	341.9	195.3
Significance	NS	NS	NS	NS	NS

Table 6.15 Microbial N content of abomasal digesta and apparent degradability of dietary N

	Microbial N content of abomasal digesta ($g\ kg^{-1}$ total N)	Uncorrected degradability of dietary N[1].	Corrected degradability of dietary N[2].
Silage E	492	0.376	0.463
Silage F	579	0.532	0.630
Dried grass	310	0.205	0.262
LSD	106.8	0.162	0.156
Significance	NS	NS	*

1. $1 - \frac{\text{(duodenal total N - microbial N)}}{\text{(intake N)}}$

2. $1 - \frac{\text{(duodenal total N - (microbial N + endogenous N))}}{\text{(intake N)}}$

Table 6.16 Dietary content of DOMR and the efficiency of synthesis of microbial N

	Proportion of DOM apparently digested in the rumen	Dietary content of DOMR ($g\ kg^{-1}$ DM)	Efficiency of synthesis of microbial N	
			($g\ kg^{-1}$ DOMR)	($g\ MJ^{-1}$ ME intake)
Silage E	0.604	392	31.3	1.15
Silage F	0.614	397	33.0	1.15
Dried grass	0.507	352	34.7	1.00
LSD	0.1365	112.7	14.5	0.432
Significance	NS	NS	NS	NS

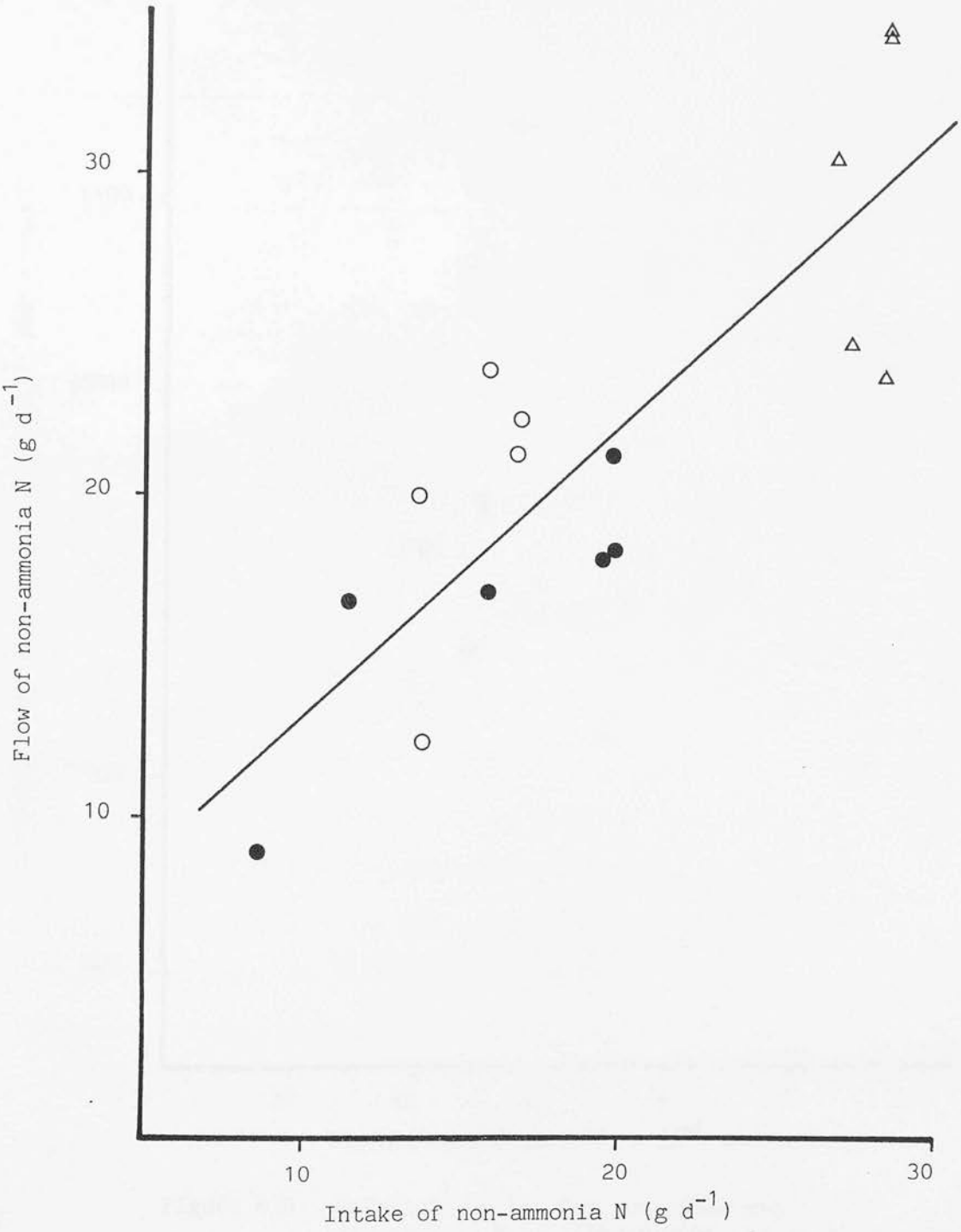

Figure 6.5 The relationship between the intake of non-ammonia N and the passage of that nutrient at the abomasum. Silage E (○), Silage F (●), dried grass (△)

Line of best fit $y = 0.9158x + 4.128$, $n = 16$, $r = 0.8406$ ($r^2 = 0.7067$)

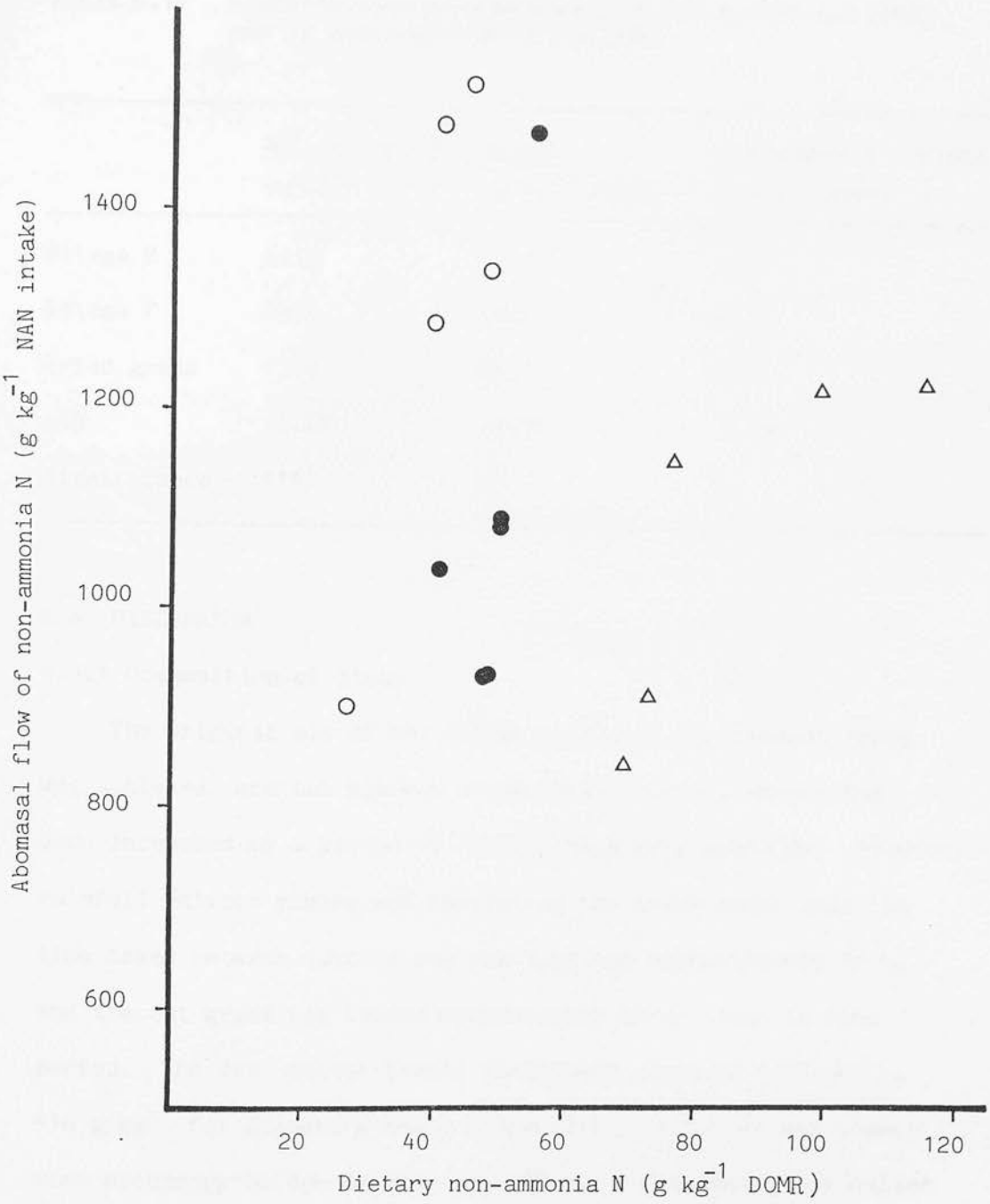

Figure 6.6 Relationship between the concentration of non-ammonia N in dietary DOMR and the flow of non-ammonia N at the abomasum. Silage E (○), Silage F (●), dried grass (△)

Table 6.17 Concentration of non-ammonia N in the DOM and DOMR, and of degradable N in the DOMR

	Non-ammonia N content		Degradable N content
	(g kg^{-1} DOM)	(g kg^{-1} DOMR)	(g kg^{-1} DOMR)
Silage E	24.2	41.9	21.5
Silage F	29.5	48.4	32.9
Dried grass	42.4	89.1	23.1
LSD	2.27	16.79	8.08
Significance	***	NS	*

6.4 DISCUSSION

6.4.1 Composition of diets

The original aim of the silage making in the present study was achieved, and two silages whose content of dry matter had been increased by a period of field drying were produced. However, rainfall between mowing and harvesting the grass meant that the time taken between cutting and ensiling was approximately 96 h, and the cut grass was tedded mechanically three times in this period. The dry matter levels ultimately achieved (438 and 416 g kg^{-1} for Silages E and F respectively) were perhaps higher than necessary to demonstrate the effects of increased dry matter on silage fermentation. The silages were drier than wilted untreated silages used in Eurowilt experiments with unwilted, additive-treated material ($\bar{x}$ = 364 g kg^{-1}), and much drier than wilted, untreated and wilted, additive-treated silages used in the same studies ($\bar{x}$ = 302 g kg^{-1}) (Zimmer and Wilkins, 1984). Silages E and F also had higher dry matter contents than the driest of

a range of silages made on a test-tube scale by Henderson and McDonald (1976) to investigate relationships between dry matter and silage ferementation. Nevertheless, the length of the wilting period which had to be used illustrates a problem of employing the technique in a west maritime climate. As has been discussed above (see Section 2.1.3.6), wilting grass, to a dry matter content of approximately 300 g kg^{-1}, is frequently advocated for minimising effluent production and increasing the pH at which a stable lactate fermentation will be established, and clostridial activity inhibited (MAFF, 1977; McDonald, 1980). Rate of drying is influenced by the extent to which the waxy surface of the leaf and stem is damaged by the mower, and 'mower/conditioners' with crimping, crushing or scarifying mechanisms incorporated are increasingly popular (MAFF, 1977). Zimmer and Wilkins (1984) concluded that wilting, with reasonably short periods of field drying, was possible throughout the areas of central, northern and western Europe included in their study. Nevertheless, the average time between cutting and ensiling in the British and Irish experiments contributing to Eurowilt (26 silages) was 46 h with a mean dry matter at ensiling of 313 g kg^{-1}. The dry matter content of the standing crop was not disclosed for all these silages, but using the value for the companion unwilted silage in each case, it appears that the rate of field drying ranged approximately from 1.0 to 8.5 g kg^{-1} h^{-1} with a mean of 3.1 g kg^{-1} h^{-1}. Thus the wilting in the present experiment, in which the rate of drying was 2.5 g kg^{-1} h^{-1}, was not untypical of that which can be expected in Britain. Notwithstanding the managerial advantages which may be derived from pre-wilting, the use of a field drying stage, on any other than

an opportunist basis, would appear to place unnecessary restrictions on a technique which should be relatively insensitive to the weather.

Another disadvantage of wilting, cited by Marsh in his review (1979), is increased loss of herbage dry matter in the field, resulting both from respiratory losses and mechanical damage. Field losses from the wilted silages prepared by the Edinburgh School of Agriculture for the Eurowilt studies were estimated as 10.7 to 12.0% (Zimmer and Wilkins, 1984). Silages E and F were made from wilted grass from one of the fields in which these assessments were made. Average loss of dry matter in the field was 8.6% in those Eurowilt experiments in which the parameter was measured (Zimmer and Wilkins, 1984). The in-field loss of dry matter for the unwilted silages made at Edinburgh in 1981 (including Silages C and D, Experiment Two) were not determined since the forage harvester followed the mower directly. Bastiman and Altman (1985) reported estimates of the in-field losses which had been made at ADAS Experimental Husbandry Farms. In preparing their data, they discarded results from six experiments in which loss had been assessed by measurements of the weight of a given length of swath, one of the two techniques used at Edinburgh. In the remaining nine studies, losses were estimated by comparing the yields of dry matter from fresh and wilted treatments cut on the same day and harvested with the same forager. The losses resulting from wilting averaged 4.8% and were negatively correlated with the content of dry matter in the ensiled herbage. This is a surprising observation, since loss of dry matter would be expected to increase with increasing length of the wilting period, and long wilting periods to be associated with drier silages. Certainly

Zimmer and Wilkins (1984) reported that the Eurowilt data confirmed earlier observations that loss of dry matter in the field was positively correlated with the dry matter content of the herbage ensiled, irrespective of weather conditions during the wilting period.

Although wilting suffers in comparison with direct ensiling because of higher field losses, the balance is at least partly redressed when losses of dry matter from the ensiled mass are considered. The mean values for the silos from which Silage C (Experiment Two) and Silage E (Experiment Three) were taken were 20.9 and 7.9% respectively. The mean values for the 21 Eurowilt experiments where this comparison was made were 16.5 and 8.6% respectively. Zimmer and Wilkins (1984) were able to fit a curvilinear function relating loss of dry matter, due to fermentation, respiration and effluent, to the dry matter content of the silage. Losses fell rapidly from about 20% with a silage of 150 g DM kg^{-1} to less than 5% at a silage dry matter of 400 g kg^{-1}. Although losses were only reported for two silages with a high dry matter, the fitted model predicted a gradual increase in dry matter loss at very high levels of silage dry matter. It is certainly the case that if wasteful aerobic respiration is to be avoided, then care must be taken to ensure adequate consolidation of ensiled herbage at high dry matter content (McDonald et al, 1976; MAFF, 1977; Thomas et al, 1980).

With the level of dry matter which was attained in each silage in the present studies, considerable restriction in the activities of clostridia and lactobacilli were expected (McDonald and Edwards, 1976; McDonald, 1980). In some respects, these expectations were

fulfilled. Silages E and F were stable and satisfactorily preserved at an appreciably higher mean pH than that of Silages C and D from Experiment Two (pH 4.32 and pH 3.75 respectively). Associated with this, some 30 - 35% of the water-soluble carbohydrate which had been ensiled was still present in the wilted silages whereas this fraction had been almost completely lost in Silage C and D. The concentration of acid products of fermentation was also lower in Silages E and F (106 and 85 g kg^{-1} DM respectively) than in the unwilted Silages C and D (159 and 125 g kg^{-1} DM respectively). In the Eurowilt studies (Zimmer and Wilkins, 1984), the mean value of total acids in unwilted silages was 20 g kg^{-1} DM higher than in the wilted group, indicating some degree of restriction of fermentation of the latter. However in these studies, and in the present experiment, the absolute levels of fermentation acids indicated that appreciable fermentative microbial activity had occurred. In those experiments in which the comparison was made, no effect of formic acid on fermentation acids could be detected when the additive was applied to wilted grass. In contrast, the present study, although allowing no statistical analysis and being confounded by different types of silo, seems to indicate that even at high levels of dry matter, heterofermentative lactic acid bacteria are active and can be partly inhibited by the addition of formic acid. Although in Silages C, D, E and F levels of butyrate were low, they were no less in the wilted than in the unwilted silages. This indicates that some clostridial activity can continue at a dry matter higher than that normally thought necessary to control that group of microorganisms (Henderson and McDonald, 1976; McDonald and Edwards, 1976; McDonald, 1980).

Other workers have observed greater influence of wilting on the degradation of carbohydrates in ensiled grass. Morgan et al (1980b) ensiled grass directly, and at a dry matter content of 365 gkg^{-1} after a wilting period of 52 h. The unwilted silage had little residual water-soluble carbohydrate and a total acid content of 193 g kg^{-1} DM. In contrast the wilted silage had a higher concentration of water-soluble carbohydrate than had the grass which was ensiled (185 and 166 g kg^{-1} DM respectively). Production of fermentation acids was greatly restricted (46 g fermentation acids kg^{-1} DM) and the pH of the silage was, in consequence, high (5.1).

As in Experiment Two, the two silages differed appreciably in their content of total N, the value for Silage F being 3.2 g kg^{-1} DM (14%) greater than that for Silage E. The level of total N was also higher in both of the silages than in the grass ensiled, in which the concentration was, in turn, higher than that of the standing crop. These differences could be ascribed to sampling errors in heterogeneous materials. However, the trend: standing crop > grass ensiled > silage, would also be produced if the dry matter lost throughout the process contained proportionally less N than did the original herbage.

There appears to have been less proteolysis in Silage E than in the formic acid-treated Silage F, although in both wilted silages the level of protein N was higher than in the unwilted silages from Experiment Two ($\bar{x}$ = 345 and 513 g kg^{-1} total N for Experiments Two and Three respectively). During successful ensiling, proteolysis results mainly from the activities of plant enzymes (McDonald, 1980). The optimum pH for plant leaf proteases is in the range

5.0 - 6.0 and as the pH of the silage falls, so their activity is reduced. Early work (Macpherson, 1952) concluded that at pH 4.3, proteolysis was prevented. However, more recent studies at the Edinburgh School of Agriculture have shown that appreciable plant enzyme-catalysed proteolysis can persist at pH 4.0, and that at lower values some acid hydrolysis can occur (McDonald, 1982). Wilting rapidly to a dry matter content >400 g kg^{-1} can severely inhibit proteolysis (McDonald, 1982), and Henderson et al (1982) demonstrated a positive relationship between the dry matter content of ensiled herbage and the protein N content of the resulting silage. The average protein N content for the silages in the current study, 513 g kg^{-1} total N, is only a little higher than the value of 447 g kg^{-1} total N reported by Henderson et al (1982) for slightly wetter materials (400 g DM kg^{-1}). The effects of wilting do not appear to be consistent, and in the experiment reported above Morgan et al (1980b) found that the protein N content of their unwilted and wilted silages was identical (286 g kg^{-1} total N). The fact that the degradation of carbohydrates and protein in ensiled herbage can be differentially affected by wilting perhaps serves to emphasise that two different agents, anaerobic bacteria and plant proteases respectively are responsible for the processes. These agents are susceptible in different ways to reduced pH and increased osmotic pressure. It is noteworthy that the wilted silage of Morgan et al (1980b) had a high dry matter (359 g kg^{-1}) but also a high pH (5.1). Silages E and F from the present study, together with those of Henderson et al (1982) combined higher dry matter with a much lower pH; factors which could act in concert to reduce the activity of plant proteases.

The levels of butyric acid and ammonia N are, in absolute terms, low (see Table 2.1). Nevertheless, comparison with the values in Table 5.2 indicates that the considerable increase in osmotic pressure resulting from wilting has not further reduced the already low level of clostridial activity in the fresh silages. Indeed, although no statistical significance can be ascribed to the differences, the butyric acid and ammonia N levels were 50% and 30% higher respectively in the wilted than in the unwilted silages. Conventionally, it would be expected that clostridial activity would be abolished at dry matter levels above 300 g kg^{-1} (McDonald, 1980; 1982). Certainly, if clostridia are active in an unwilted silage, then a wilted companion is likely to show lower levels of butyric acid and ammonia N (Henderson and McDonald, 1976; Morgan et al, 1980b). However when an acid or acid/formalin additive is applied to the unwilted grass, the wilted 'partner', whether treated with an additive or not, may frequently contain more ammonia N. Wilted silages made at Liscombe Experimental Husbandry Farm over a period of three years (MAFF, 1981; 1982; 1983) consistently demonstrated higher ammonia N contents than did unwilted controls. In several instances the ammonia N levels were high enough to classify the silages as 'bad' or 'unsatisfactory' (McDonald and Whittenbury, 1973).

6.4.2 Ruminal metabolism

Intakes were uniform, and not statistically different from the target of about 1 kg DM d^{-1}, during the period when ruminal metabolism was investigated. It is at once apparent from Tables 6.5 and 6.6, together with the corresponding Figures 6.2 and 6.3, that neither the ammonia N content nor the pH of rumen liquor

was influenced by the use of formic acid as a pre-ensiling additive. As in Experiment Two, the utilisation of silage N was, on first inspection, much less wasteful than that of dried grass, with peak ruminal ammonia N concentrations of 219 and 238 mg l^{-1} for Silages E and F respectively compared with a value of 341 mg l^{-1} for the dried grass. However, by taking account of the content of total N in the diet, and of the magnitude of the ammonia N peak generated from the pre-feeding baseline a different picture emerges. The resulting values, with units mg ammonia N l^{-1} g^{-1} N intake, are 7.74, 6.07 and 4.98 for Silages E and F and dried grass respectively. The nitrogenous components in the silages now appear more susceptible to degradation than do those of the dried grass and these figures can be usefully compared with the degradability figures reported below (see Section 6.4.4). It is clear when comparing Fig 6.2 with Fig 5.2 and Fig 6.3 with Fig 5.3 that the diurnal variation of both parameters was very similar for the dried grass diet in both experiments. It therefore seems valid to make comparisons between the experiments. Although the relative positions of the unwilted silages and the dried grass were altered in Experiment Two when ammonia N peaks were expressed as mg g^{-1}N intake, the changes were less marked than those in Experiment Three. The wilted silages from the present experiment 'generate' ruminal ammonia peaks 17 - 150% higher per unit N intake than did their unwilted companions from the earlier experiment. The consequences of these differences do not appear to include less efficient synthesis of microbial N in the rumen (see Section 6.4.4 below). Nevertheless, Hinks and Henderson, when reporting Eurowilt studies at Edinburgh which used Silages D and E (Zimmer

and Wilkins, 1984), stated that the N fraction of the wilted silage was less well utilised by growing lambs than was that of the direct-cut material. This conclusion was reached because lambs fed Silage D and barley showed no response in liveweight gain to supplementation with soyabean meal. In contrast supplementing Silage E and barley with the protein source increased average liveweight gain over a nine week period by 35 g d^{-1}. When considering possible differences between the silages from Experiments Two and Three, in the production of ruminal ammonia N, the caveat that rates of dry matter intake were higher for the wilted than for the unwilted silages, must be added. No quantitative measurements were made, but although the total intakes were probably not statistically different, the wilted material was obviously more palatable to the particular animals used in these experiments. That which was eaten was consequently consumed more rapidly.

In contrast with the unwilted silage (see Fig 5.4), pre-ensiling treatment with formic acid did not greatly alter the NGR of the rumen volatile fatty acids when the silages were fed to sheep. Except for a short period immediately after feeding, the ratio for both silages was above the range identified by Ørskov (1975) as being associated with most efficient use of dietary energy for fattening (2.25 - 3.0). McDonald and Edwards (1976) suggested that a trend in NGR of wilted silage > lactate silage > formic acid-treated silage might occur. The results of Experiments Two and Three do not support this, suggesting, if anything, a trend in the opposite direction.

6.4.3 Dietary digestibility

Intakes of silage dry matter during the digestibility trial did not differ significantly between diets and could not be distinguished from the target intake of about 1 kg DM d^{-1}. Although the sheep used in the experiment spanned a range of body weights, and yet were all offered the same quantity of food, the mean intakes recorded were close to the predicted appetite of 0.92 kg DM d^{-1} for sheep of the same average weight (ARC, 1980). Because the intakes recorded in the present series of experiments were, despite apparent restrictions in food supply, close to the maximum appetite predicted by the Agricultural Research Council (ARC, 1980), the validity of the Council's predictions may be questioned. In experiments in which the measurement was given, sheep in the Eurowilt experiments (Zimmer and Wilkins, 1984) consumed, on average 48.4 g DM $kg^{-0.75}$ d^{-1} of wilted silage, a figure 5% greater than the value of 46 g $kg^{-0.75}d^{-1}$ adopted by the ARC. The Eurowilt studies also demonstrated higher intakes of wilted than unwilted silage for dairy cows, growing cattle and sheep, although in all cases the increase was less than 10%. The mean value for Silages E and F in the present study (45.1 g DM $kg^{-0.75}$ d^{-1}) was about 18% higher than that of Silages C and D in Experiment Two (38.1 g DM $kg^{-0.75}d^{-1}$).

The Eurowilt studies, encompassing 36 experiments with several classes of three ruminant species, probably represent the most reliable database of the effects of wilting on silage intake. Nevertheless, it must be appreciated that the mean values for these experiments conceal several individual instances in which no effect was observed or the difference was in the opposite

direction. In other experiments with cattle at Liscombe Experimental Husbandry Farm (MAFF, 1981; 1982; 1983) the intake of wilted silage was greater than that of unwilted silage in the first two years whereas in 1983 there was a slight benefit (5%) in favour of the fresh material. Neither Donaldson and Edwards (1976) nor Morgan et al (1980b) could demonstrate any significant response, in voluntary intake, to wilting ryegrass before ensiling. Since the voluntary intake of dry matter may be inversely related to the concentration of fermentation products in the silage, it is possible that this mechanism may explain the increases seen with wilted silages (McDonald andEdwards, 1976). The fact that restriction of fermentation seems to be very variable in wilted silages, and only in part dependent on the dry matter content of the material, may in turn explain why the observed effects of wilting are not constant.

Tables 6.8, 6.9 and 6.10 show once again that the differential treatment had no significant effect on the parameters measured in the digestibility trial. The apparent digestibility of total N, and consequently the content of DCP, was higher for Silage F than for Silage E. Unfortunately, as has been discussed above (see Section 4.2.2, 5.4.3), apparent digestibility of N is confounded with the level of the nutrient in the diet. In addition, comparison between the digestibility coefficients from this experiment and those from Experiment Two , indicates that the effects of wilting per se have been slight. There is perhaps a suggestion that the digestibilities of organic matter and gross energy are slightly lower for the wilted than for the unwilted silage (0.704 vs 0.723 and 0.672 vs 0.701 respectively), but in all cases the 95%

confidence interval for the parameter in one experiment embraces the mean value for the same variable in the other. Morgan et al (1980b) found that wilting significantly ($P < 0.05$) reduced the digestibility of the organic matter in silage when fed to cattle, but not when fed to sheep. These authors also observed a non-significant increase in the apparent digestibility of total N in the (unwilted) silage with a higher content of the component.

The review of the Eurowilt experiments (Zimmer and Wilkins, 1984) is critical of the fact that the contributing experiments included so few in which the digestibility of the silages was determined in vivo. For those in which this was done, a similar trend to that observed in the present experiment was apparent, with mean values of 0.705 and 0.740 for the digestibility of organic matter in wilted and unwilted silages respectively. Zimmer and Wilkins (1984) concluded that this difference would largely offset the slight advantage in dry matter intake of the wilted materials. The measurements in vitro of digestible organic matter content reported for other experiments in the same series did not, in contrast, show any trend in this direction, the mean value for wilted silages (661 g kg^{-1} DM) being slightly greater than that for the unwilted controls (640 g kg^{-1} DM).

Urinary excretion of N, as a percentage of N consumed, did not differ between Silages E and F, nor between the silages and the dried grass. The 95% confidence limits of the mean values in the present study (22.8 - 58.6% and 27.1 - 63.9% for Silages E and F respectively) do not encompass the mean value for the unwilted silages in Experiment Two (65.7%). However, the variation associated with the measurements in Experiment Two would make

unequivocal comment on biological differences, between the two types of silages, unwise.

With the type of animal being used, nominally mature wethers of various weight and ages, the usefulness of the observed N retention figures in ascribing differences to the efficiency of utilisation of dietary N must be questioned. However the standard dried grass diet gave mean values of 7.3, 6.0 and 6.7 g N d^{-1} in Experiments One, Two and Three, suggesting that, despite their heterogeneous makeup, the groups of sheep responded in a fairly uniform manner. The mean values reported for the wilted silages here are clearly higher than those for the unwilted material in Experiment Two. Indeed the 95% confidence limits for Silage F (2.97 - 7.27) and Silage C (-2.67 - 2.67) do not overlap at all. In addition, for the first time in the present series of experiments, the value for a silage (Silage F) was statistically indistinguishable from that of the dried grass.

These observations were apparently contradicted when Silages D and E were fed to growing lambs at the Edinburgh School of Agriculture (Zimmer and Wilkins, 1984). As has been stated above (see Section 6.4.2), the nitrogen fraction of the unwilted silage was more efficiently used for liveweight gain than was that of the wilted material. Few measurements of retention of energy or N were made in the Eurowilt experiments. In two, in which the composition of the liveweight gain was assessed, there was a tendency for a higher proportion of fat to be laid down in the carcass of animals fed unwilted, additive treated, rather than wilted, silage. This difference could conceivably allow greater liveweight gain, but poorer retention of N, with the former than with the latter.

6.4.4 Rate of flow of digesta

Intake of dry matter, at the times during the experiment when rate of flow of digesta was being determined, were high and showed no significant differences between diets. However, a trend which was perhaps developing for Silage F and the dried grass during the digestibility trial appeared to continue towards the end of each experimental period. It seems unlikely that this reduced intake, after consuming the diet for four or five weeks, would be a characteristic apparent in commercial feeding. Indeed many animals eating silage on the farm are actively growing and absolute intake would be expected to increase with time. However the metabolism studies reported here use relatively few animals, familiarised with a highly artificial environment and with a standardised diet of hay and rolled oats when not being used for an experiment. That the confinement imposed by the metabolism cages can be stressful is illustrated by the stereotyped behaviour of individual sheep (Woodgush et al, 1975; see Section 5.2.1), and reduced voluntary intake of food might be expected as a consequence of this stress.

Once again the sign of the reconstitution factors was negative, indicating a sampling bias towards the particulate phase of digesta. This was seen in Experiments One and Two and has been reported by other workers (Faichney, 1975b; Beever et al, 1978). The random variation associated with this measurement was high and as a result the 95% confidence intervals for R embraced zero for all three diets. Thus, using conventional standards of statistical surety, the digesta samples collected during this experiment could not be distinguished from truly unbiased ones. However such an

observation would provide an unsound basis from which to challenge the sound theoretical, and frequently-demonstrated practical reasons for using a complex dual-phase marker with simple intestinal cannulas.

The average recoveries of Ru and Cr were slightly poorer than those reported in Experiment Two, although not as low as those in Experiment One. As was discussed in Section 4.4.3, the fact that measurements of total flow of Ru and Cr were made only at the point of infusion and in the faeces, makes the assessment of the impact of poor recovery on the measurements of rate of flow of abomasal digesta difficult.

Few significant differences were apparent in the various parameters determined in this section of the experiment and presented in Tables 6.13 - 6.17. In contrast with Experiment Two, however, this did not mean that 'true' differences were being masked by unacceptably high random variability in the observations. Whereas coefficients of variation for these measurements with the unwilted silages ranged from 17 - 168%, the same measurements with Silages E and F had coefficients in the range 8% to 49%. Experiment Three was conducted using the same methods and materials as was Experiment Two and no reason can therefore be given for one yielding far more precise results than the other.

None of the nutrient flows shown in Table 6.13 showed a significant difference between the untreated and formic acid-treated silages. This is noteworthy in the case of flow of total N and non-ammonia N where the consumption of a similar amount of a silage with a higher concentration of the fractions (Silage F) did not increase the flow compared with that of Silage E. The direct effects of wilting, assessed by comparing Experiments Two and

Three, can only be discussed in general terms since none of the differences are large enough to escape the very large 95% confidence limits of the mean values in Experiment Two. However, one would have confidence that the flow of microbial N did really differ between the wilted and unwilted materials since the mean value for Silages E and F was about 50% greater than that for Silages C and D.

The values for flow of total N (g kg^{-1} total N intake) in Table 6.14 exhibit a trend which is consistent with the theory that the value varies inversely with the concentration of total N in the diet. Such a relationship has been demonstrated clearly for dried and pelleted forages by Thomas (1982) and the collected observations from Experiments One to Four suggest that a similar relationship exists for grass silages (see Section 8, Fig 8.2). Although there are non-significant ($P > 0.05$) differences between Silages E and F, and also differences between the wilted and unwilted groups it seems more likely that these reflect the differences in total N content of the diets rather than true effects of the pre-ensiling additive or wilting treatments.

All three parameters in Table 6.15 demonstrate a similar relationship between the two silages, indicating greater breakdown of the nitrogenous fraction of the diet, and hence more microbial N in the abomasal digesta, for Silage F than for Silage E. Even when formic acid influences the composition of the nitrogenous fraction of silage, its effects on the degradation of that N in the rumen appear to be relatively minor (Siddons et al, 1982; McDonald et al, 1983). In the present study, formic acid had very little effect on the composition of the silage, indeed the

concentration of protein N was higher in the untreated Silage E. The higher degradability of dietary N for Silage F is difficult to explain. It could, conceivably, result from the lower level of protein N, although the results of Mcdonald et al (1983), showing that protein N which was not chemically protected was only slightly delayed in its pasage from Dacron bags incubated in the rumen, do not support this explanation.

In the present experiment, the corrected degradability values are as high as, or higher than, those from Experiment One. The 95% confidence interval for Silage F (0.47 - 0.79) reaches the mean value of 0.80 selected by the Agricultural Research Council for a range of foods including wilted and unwilted silage (ARC, 1980). In contrast, the same intervals for Silage E and the dried grass do not reach the lower end of the ranges suggested (0.71 and 0.51 respectively) (ARC, 1980).

Once again, the proportion of digestible organic matter which was apparently digested in the rumen was lower, for all diets, than the mean value of 0.65 adopted by the Agricultural Research Council (ARC, 1980; 1984). Although the Council advocated the use of a single mean value, it stated that differences between types of diet existed, and the mean value quoted for pelleted, dried grass was 0.51 (ARC, 1980), the same as that observed in the present study. A mean value of 0.62 has been suggested for silages (ARC, 1980), but Thomas (1982) reported that previous work showed that the value was almost constant at 0.70 for silages. The precision of the measurements precludes an unequivocal statement that the proportion for the silages in Experiment Three is less than 0.65 or 0.70. However the fact that all the mean values

for the eight silages were less than 0.70 is persuasive circumstantial evidence that the true values are less than the 'universal' figure quoted by Thomas (1982).

The apparent efficiencies of synthesis of microbial N, shown for the silages in Table 6.16, are higher than many which have been reported elsewhere. In both cases (g N kg^{-1} DOMR and g N MJ^{-1} ME intake) the efficiency terms are higher than those reported in Experiment Two for the unwilted silages because of the greater contribution of microbial N to total N in abomasal digesta. However, in comparison with previously published values, the efficiency expressed per unit DOMR is high because modest levels of microbial N are combined with relatively low digestion of organic matter in the rumen (ARC, 1980; 1984; Beever, 1980; Thomas, 1982). Efficiency per unit intake of ME is less exceptional when compared with the previously published mean value for sheep of 1.34 (ARC, 1984). Nevertheless, the database used by the Agricultural Research Council included only five observations with grass silage as the sole constituent of the diet, and for these experiments the mean value was 0.71 g microbial N MJ^{-1} ME.

Although associated with unacceptable random variability, the mean values for the unwilted silages in Experiment Two were very close to those quoted by the Agricultural Research Council (ARC, 1980; 1984). It is thus apparent that Experiment Three demonstrates an important improvement in the efficiency of synthesis of microbial N in response to wilting. This partly conflicts with the conclusion of Beever (1980) that the efficiency of synthesis of microbial protein was lower for wilted, than for direct-cut, silage. However Beever demonstrated these differences by referring

to the apparent capture of N by rumen microbes (g 100 g^{-1} degraded dietary N), the values being 89, 69 and 89 for fresh grass, wilted silage and unwilted silage respectively. Multiplying the intake of total N by the corrected degradability of dietary N in the present experiments gives the quantity of N apparently degraded in the rumen. This value, combined with the flow of microbial N at the abomasum, allows computation of the parameter quoted by Beever. The mean values are 157 and 124 g microbial N 100 g^{-1} degraded N for unwilted and wilted silages in Experiments Two and Three respectively. These figures demonstrate exactly the trend shown by Beever (1980). Unfortunately, the values for dried grass in Experiments Two and Three were 80 and 148 g microbial N 100 g^{-1} degraded N, indicating that the parameter varied more for one diet between experiments than between the different classes of silage.

Beever (1980) attempted to relate variation in the efficiency of synthesis of microbial N to differences in the ratio of degraded N to degraded carbohydrate in his silages. Silages E and F did have significantly different ratios of degraded N to degraded organic matter, that for the formic acid-treated material being higher than that of the control ($P < 0.05$, Table 6.17). However, there were no differences between these in the efficiency of synthesis of microbial N. Although the usual caveat on large random variability must be given, a large difference was observed between the mean values for the unwilted silages in Experiment Two (13.3 g degradable N kg^{-1} DOMR) and the wilted silages in the current study (27.2 g degradable N kg^{-1} DOMR). It is therefore tempting to suggest that as the ratio of substrate providing N for microbial synthesis, to that providing the energy, increases, the efficiency

of the synthetic processes rises. This contradicts the findings of Beever (1980) who found that the ratio of degraded N to degraded carbohydrate was lower in the diets with high apparent capture of degraded N.

The scatter diagram of intake and flow of non-ammonia N (Fig 6.5) shows a positive linear relationship existing between the two. Thomas (1982) found a similar relat ionship when reviewing previous experiments with silages although the equation describing the straight line, $y = 1.11x + 1.36$, indicated a slightly steeper gradient and a smaller y-axis intercept than were found in the present studies. This intercept, the flow of non-ammonia N when N intake is zero, may provide an estimate for the amount of endogenous N flowing in the digesta at the point of sampling. The estimate from the equation of Thomas (1982) agrees reasonably with previously published values (Smith et al, 1976; Lindsay and Armstrong, 1982) whereas the value of 4.13 g non-ammonia N d^{-1} derived from Fig 6.5 is considerably in excess of most estimates.

From Fig 6.6 it is clear that the ratio of non-ammonia N to DOMR in the diets had no overall effect on the passage of non-ammonia N at the abomasum. Significant positive relationships can be fitted, to the observations of the two silages, but the slopes are very steep and the lines closely approach the vertical, at which point they are of no predictive value.

6.5 CONCLUSIONS

Experiment Three emphasised that although field drying can be used to increase the dry matter content of herbage before ensiling, the process may take much longer than the time required when the weather is clement. The advantages of the use of an unnecessary, weather-dependent, step in any system for making silage must be carefully considered before the step is adopted.

As in Experiment Two, there were indications in the present study that factors other than the imposed pre-ensiling treatments may have influenced the composition of the silages. No prounounced effects of wilting or formic acid on the level of total N in silage have been reported by other workers, and it is therefore likely that the differences observed in the silages resulted from ensiling relatively heterogeneous material.

The present study confirmed the restrictive effect of wilting on proteolysis and the fermentation of carbohydrates in silage. However, even at the levels of dry matter used, which were higher than is common in commercial practice in the United Kingdom, appreciable heterofermetative activity did occur. Wilting, with or without the support of formic acid, does not appear to curtail the low levels of clostridial activity which occur in well preserved, unwilted, silages.

The trend shown for production of ammonia N in the rumen in Experiment Two was confirmed with the wilted silages in the present study. Specific production of ammonia N (per unit intake of total N) was higher for the wilted silages than for the dried grass. The rate of intake of dry matter probably influences this parameter, but specific production of ammonia N appears to be greater for wilted than for unwilted silages.

Extensive ruminal degradation of the nitrogenous fraction of wilted silages was confirmed in this experiment. Despite the resulting high ratio of degraded N to degraded organic matter in the diet, the apparent efficiency of synthesis of microbial N was higher than the average of previously published estimates for silages.

Intake of non-ammonia N proved to be an adequate estimator of the flow of the nutrient at the abomasum for all diets. This confirms the findings of the Agricultural Research Council, and must therefore carry the same warning; such a simple relationship can only have a limited application taking, as it does, no account of all the known dietary factors which may influence the supply of N to the small intestine.

7. EXPERIMENT FOUR

7.1 INTRODUCTION

The intensive management of grassland for silage making has resulted in increased use of the regrowths from swards cut in the Spring and early Summer (MAFF, 1977). Apart from the greater total quantity of fodder which can be conserved for winter feeding, the technique has the potential to produce silage with high contents of digestible nutrients. For a ryegrass sward, the slow fall in the digestibility of the herbage with time means that a period of about six weeks regrowth can be allowed before the sward has declined to the condition of a first cut taken a few days after ear emergence. However the higher content of total N and lower content of water-soluble carbohydrate in the ensiled grass make the attainment of stable conditions in the silage less likely, particularly if pre-wilting is not used (Henderson and McDonald, 1976; Adamson and Appleton, 1984).

If a weather-dependent wilting step is not to be introduced into the silage making process, an additive may be applied to ensure the safe preservation of the crop. The effects of formalin, in suppressing the fermentation of ensiled herbage and reducing the degradation of herbage nitrogenous components in the silo and in the rumen, are well documented (Wilkins et al, 1973). However at low application rates, suppression of clostridia may be inadequate (Wilkins et al, 1973) and if sufficient is used to avoid these problems the protein may be overprotected, thus negating any nutritional benefit from reduced ruminal degradation (Barry, 1973b). There is also concern that formaldehyde, present at high concentrations in feed, may be transferred to the animal products which are eaten by humans (Kreula and Rauramma, 1976). The use of lower

levels of formalin in combination with mineral or organic acids, combining some protection of protein with a stable fermentation, has therefore been advocated (Castle et al, 1977; Hinks and Henderson, 1977; Donaldson and Edwards, 1979; 1980). The greatest success has been achieved with combinations of formalin and formic acid in which the latter predominates.

In the present study, the composition and nutritional value of ryegrass silage, untreated or treated with formic acid and formalin, and made from the primary regrowth of the sward used in Experiments Two and Three, was investigated.

7.2 METHODS AND MATERIALS

7.2.1 Experimental design and procedure

The animal studies of Experiment Four were conducted between 18 April and 31 July 1983. The three experimental periods were each of 5 weeks' duration and followed the format described in Section 3.2 above.

Six sheep, numbers 37, 41, 91, 487, 510 and 1280 were used in this experiment. The average weight of these animals was 60.0 kg with a minima and maxima of 45.0 and 76.4 kg. The allocation of diets for each animal is shown in Figure 7.1.

The only disruption to the intended schedule occurred towards the end of the third period, when no abomasal digesta could be obtained from Sheep 510. There were therefore only five sets of estimates for the various parameters of rate of flow from the pelleted, dried grass.

	Column					
	I	II	III	IV	V	VI
Sheep	37	1280	510	41	91	487
Period						
1	G	DG	H	G	DG	H
2	DG	H	G	H	G	DG
3	H	G	DG	DG	H	G

Figure 7.1 Allocation of three diets to six sheep over three experimental periods in Experiment Four. Untreated silage (G), silage treated with formic acid and formalin (H) and pelleted, dried grass (DG)

7.2.2 Diets

Three diets were offered in Experiment Four, two grass silages and the pelleted, dried grass which was fed in each experiment.

7.2.2.1 Silages

Both silages in the present study (G and H) were made from the same sward, in July 1981. The grass used was a primary regrowth from the area which had provided the grass for Experiments Two and Three. The botanical composition of the sward was assessed on 26 July 1981. Italian and perennial ryegrass each accounted for between 30 and 60% of the sward, with appreciable variation in their relative abundance across the field. White clover (Trifolium repens) contributed about 5% to the total over the whole site. The standing crop was sampled on the same day and its content of dry matter and total N determined.

The crop was cut with a drum mower on 28 July and lifted with a precision-chop forage harvester on the same day. About 5 t of harvested material was ensiled without further treatment in a concrete bunker silo 1.6 m wide, 2.8 m long and 1.6 m deep. The material was consolidated manually at frequent intervals as the silo was

being filled. When this was completed, the grass was covered with polythene sheeting which was sealed and weighted with sandbags. A second silo was similarly filled with about 5 t of grass, which was treated with a mixture of formic acid and formalin as the silo was being filled. The additive, which contained formic acid (Add-F, BP Nutrition (UK) Ltd) and formalin (a 38% w/v solution of formaldehyde) in the proportions 4:1, was applied at the rate of 4.5 l t^{-1}, using a watering can. Periodically, the solution was spread evenly over the ensiled material after a thin layer of grass had been placed in the silo and consolidated. The additive-treated surface was then covered with more grass which was consolidated before any more additive was applied.

The silo containing untreated silage (Silage G) was opened on 16 October, and that containing the treated material (Silage H) on 19 October 1981, after ensiling periods of 81 and 84 d respectively. For each, surface spoilage was removed and approximately 1.5 t of silage was placed on a clean concrete floor where it was thoroughly mixed. The silage was representatively sampled for subsequent analysis (see Section 3.4.1) and 210 polythene bags were each filled with 6 kg of silage. This provided slightly in excess of the required daily ration of 1 kg dry matter. The bags were sealed and then stored at -20°C until required. Silage was thus in refrigerated storage for between 544 and 651 d before being thawed and fed to the sheep.

7.2.2.2 Dried grass

The pelleted, dried grass was stored in a pest-proof container. At intervals during the course of the experiment pellets were weighed into polythene bags containing the daily ration of 1.14 kg (1 kg dry matter).

7.3 RESULTS

7.3.1 Composition of diets

The composition of the standing crop and the grasses which were ensiled are presented in Table 7.1. The compositions of the resulting silages are shown in Table 7.2 and that of the pelleted, dried grass in Table 7.3.

Table 7.1 Composition of standing crop and grass as ensiled

Component ($g\ gk^{-1}$ DM unless otherwise stated)	Standing crop	Grass as ensiled	
		Silage G	Silage H
Dry matter ($g\ kg^{-1}$)	181	179	176
Total N	27.4	26.5	25.4
Water-soluble carbohydrate	-	165	175
MAD fibre	-	285	278

Table 7.2 Composition of silages

Component (g kg^{-1} DM unless otherwise stated)	Silage G	Silage H
Dry matter [1] (g kg^{-1})	192	188
Dry matter [2] (g kg^{-1})	207	197
pH (units)	3.86	3.91
Organic matter	908	910
MAD fibre	304	311
Water-soluble carbohydrate	0	64
Ethanol	6.7	9.5
Lactic acid	110.0	66.0
Acetic acid	29.4	21.8
Propionic acid	0.3	0.4
n-Butyric acid	0.1	0.1
Formic acid	0.8	21.9
Total N	26.0	24.0
Protein N	11.8	12.5
Protein N (g kg^{-1} total N)	452	522
Ammonia N	1.9	1.0
Ammonia N (g kg^{-1} total N)	75.0	42.0
Non-ammonia N	24.1	23.0
Non-ammonia N (g kg^{-1} total N)	927	958
Gross energy (MJ kg^{-1} DM)	19.2	18.9

1. Determined by drying to constant weight at 100°C

2. Determined by distillation with toluene (see Section 3.4)

Table 7.3 Composition of dried grass

Component (g kg^{-1} DM unless otherwise stated	Pelleted, dried grass
Dry matter [1.] (g kg^{-1})	878
Organic matter	933
Water-soluble carbohydrate	190
Total N	28.5
Protein N	23.4
Protein N (g kg^{-1} total N)	821
Ammonia N	0.3
Ammonia N (g kg^{-1} total N)	11.0
Non-ammonia N	28.2
Non-ammonia N (g kg^{-1} total N)	989
Gross energy (MJ kg^{-1} DM)	18.8

1. Determined by drying to constant weight at 100°C

7.3.2 Ruminal metabolism

Intakes of dry matter, recorded during the period when ruminal metabolism was being monitored, are shown in Table 7.4. There were no differences of statistical significance between the diets. Diurnal variation in the concentration of ammonia N in strained rumen liquor, and in the pH of rumen liquor, are presented in Tables 7.5 and 7.6 respectively. The relationships are shown graphically in Figures 7.2 and 7.3. The magnitude of the initial rise in ammonia N concentration, after feeding, was greater for Silage G than for Silage H ($P < 0.05$). The high level of ammonia N was also more persistent, with the concentrations at 15.00 h

($P < 0.05$), 17.00 h and 21.00 ($P < 0.01$) being greater for the untreated than for the additive-treated silage. The values of pH of rumen liquor at 07.00 h are arithmetic means of readings taken in Periods 1 and 3 only. Feeding Silage G instead of Silage H resulted in a significantly higher pH of rumen liquor for at least 8 h after the food was offered.

Diurnal variation in the non-glucogenic ratio of the rumen volatile fatty acids is illustrated in Figure 7.4. The patterns for each silage were very similar, showing a pronounced fall in the ratio in the first hour after fresh food was offered, followed by a steady return to the pre-feeding value over the next 23 h. The NGR when dried grass was fed was, at all sampling times, higher and the fall and rise which followed feeding were compressed into a period of 6 - 12 h.

Table 7.4 Amount of dry matter consumed during measurement of ruminal metabolism

	Dry matter intake		
	$g\ d^{-1}$	$g\ kg^{-1}\ LW\ d^{-1}$	$g\ kg^{-0.75}\ LW\ d^{-1}$
Silage G	945	16.1	44.5
Silage H	1117	18.6	51.6
Dried grass	985	16.5	45.8
LSD	175	2.94	8.1
Significance	NS	NS	NS

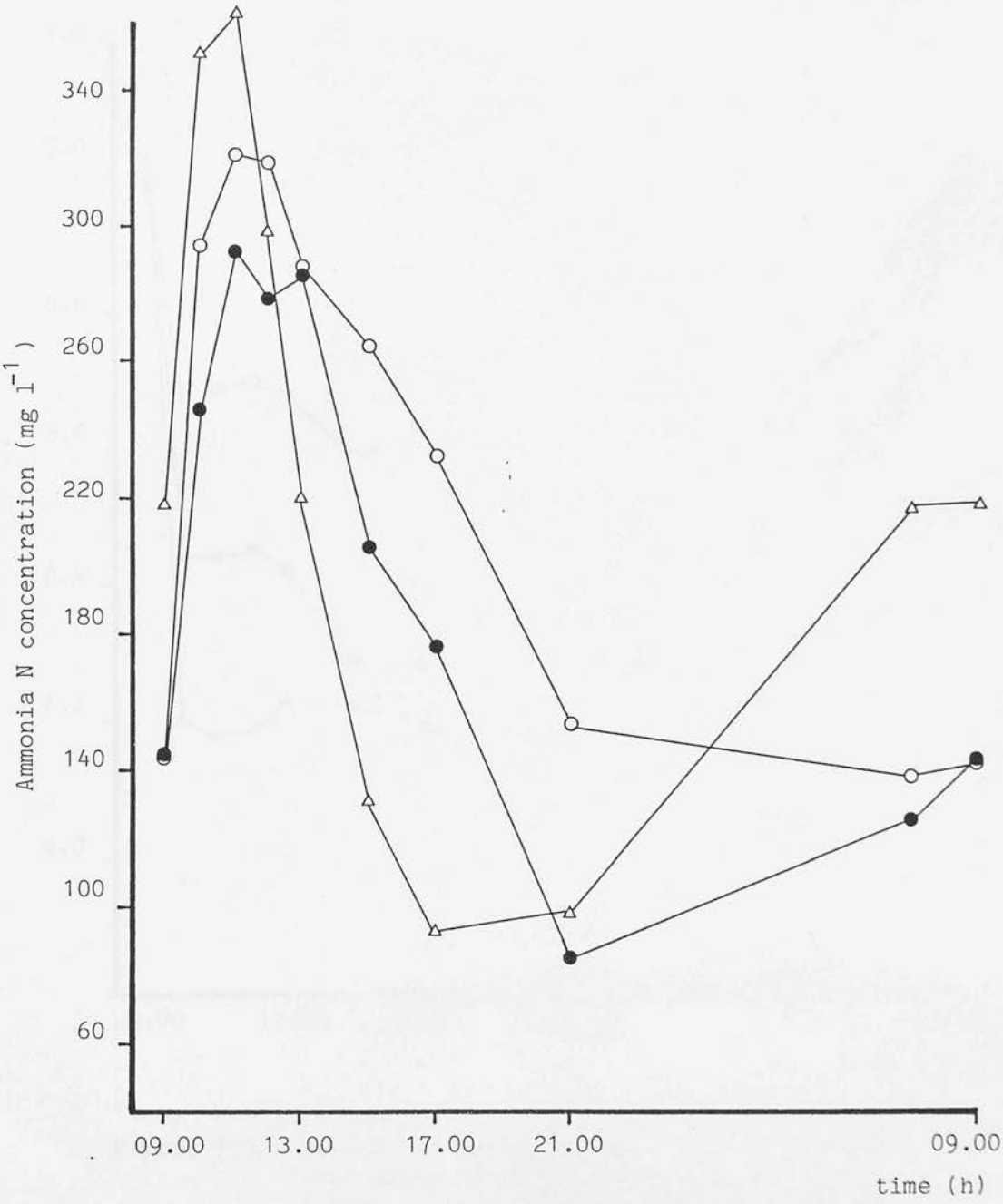

Figure 7.2 Diurnal variation in the concentration of ammonia N in strained rumen liquor from sheep fed once daily at 09.00 h. Silage G (○), Silage H (●), dried grass (△)

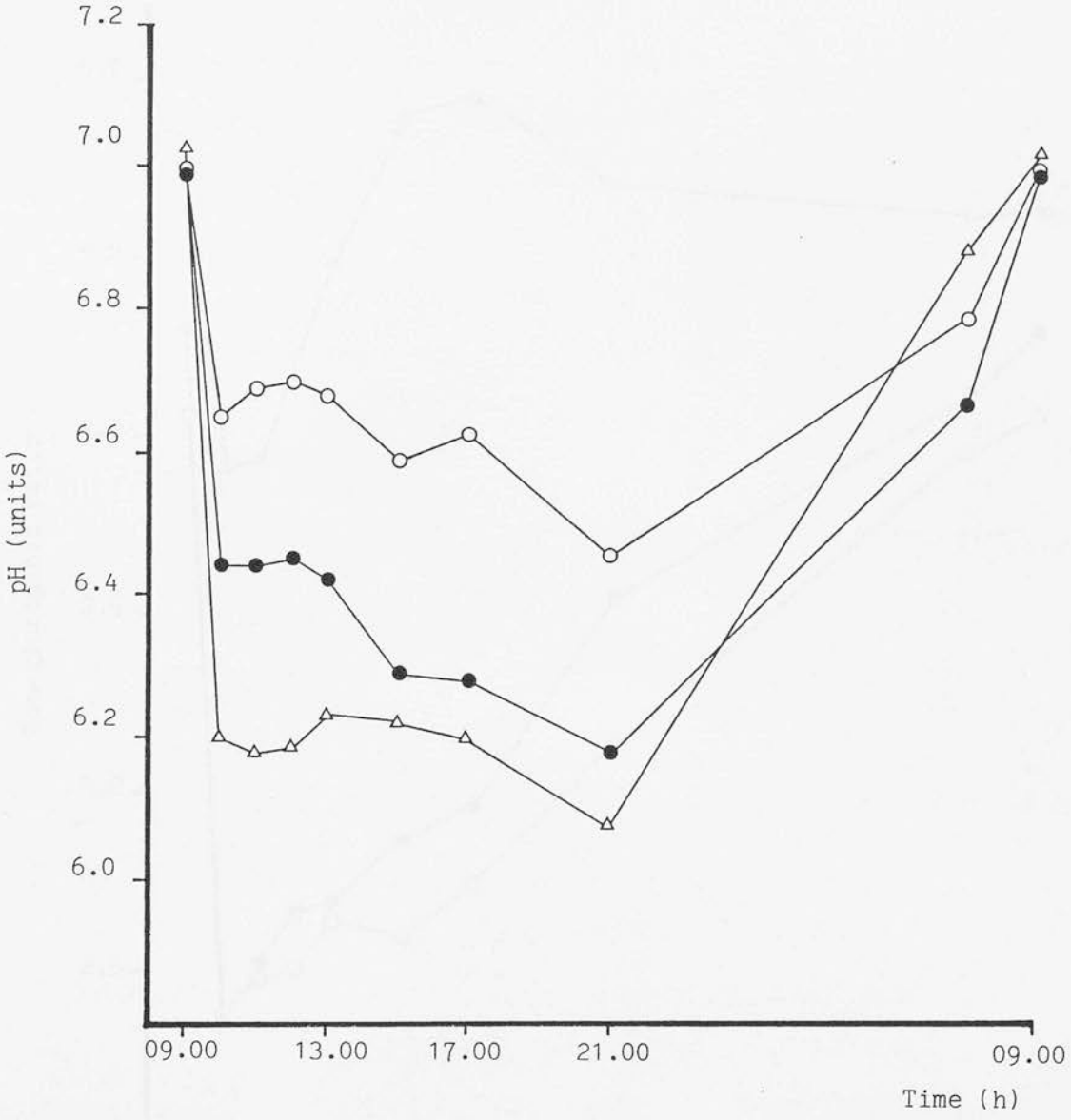

Figure 7.3 Diurnal variation in the pH of rumen liquor from sheep fed once daily at 09.00 h. Silage G (○), Silage H (●), dried grass (△)

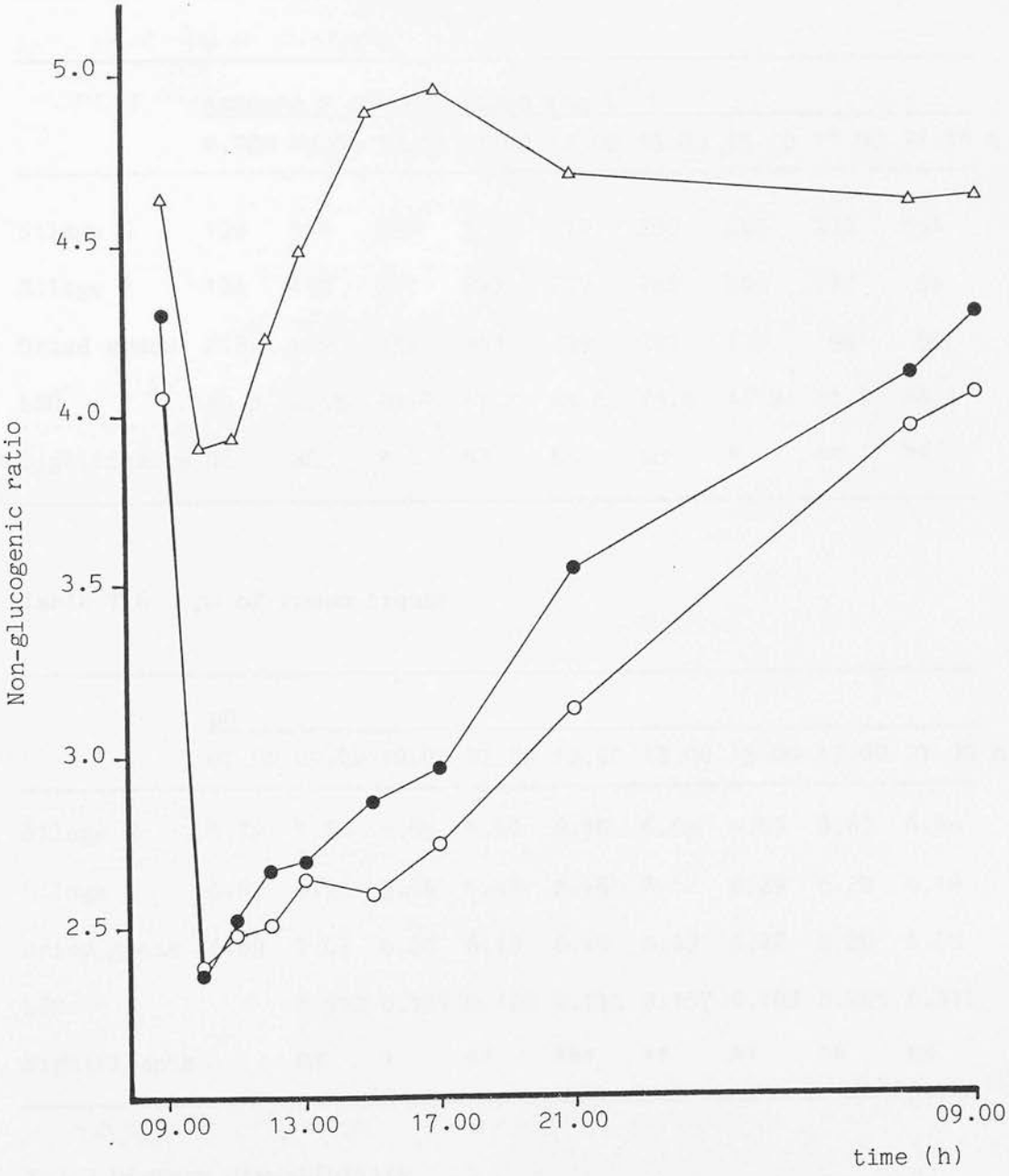

Figure 7.4 Diurnal variation in the non-glucogenic ratio of the volatile fatty acids in strained rumen liquor from sheep fed once daily at 09.00 h. Silage G (○), Silage H (●), dried grass (△)

Table 7.5 Concentration of ammonia N in strained rumen liquor

	Ammonia N concentration (mg l^{-1})								
	0.700	09.00	10.00	11.00	12.00	13.00	15.00	17.00	21.00 h
Silage G	139	144	295	322	319	289	265	233	154
Silage H	126	145	247	293	279	286	206	177	86
Dried grass	218	219	352	363	299	221	132	94	99
LSD	43.8	23.5	39.9	47.7	49.6	75.6	42.9	31.8	46.1
Significance	NS	NS	*	NS	NS	NS	*	**	**

Table 7.6 pH of rumen liquor

	pH								
	07.00	09.00	10.00	11.00	12.00	13.00	15.00	17.00	21.00 h
Silage G	6.79	7.00	6.65	6.69	6.70	6.68	6.59	6.63	6.46
Silage H	6.67	6.99	6.44	6.44	6.45	6.42	6.29	6.28	6.18
Dried grass	6.89	7.03	6.20	6.18	6.19	6.23	6.22	6.20	6.08
LSD		0.312	0.167	0.128	0.113	0.157	0.183	0.205	0.311
Significance		NS	*	**	***	**	**	**	NS

7.3.3 Dietary digestibility

The amounts of dry matter consumed during the period when dietary digestibility was measured, are shown in Table 7.7. A combination of increased intake of Silage H, together with less variability in the measurements (average cv% = 15 and 22% for intake measurements in Tables 7.7 and 7.4 respectively) meant that during the digestibility trials, the intake of Silage H

was significantly higher than that for Silage G ($P < 0.05$). The results of the measurements collected during this phase are presented in Tables 7.8 - 7.10. The digestibility of total N was higher for Silage G than for Silage H ($P < 0.05$), with the result that the DCP content of Silage G was 12% greater than that of the silage treated with formic acid and formalin ($P < 0.001$).

Table 7.7 Amount of dry matter consumed during measurement of dietary digestibility

	Dry matter intake		
	g d^{-1}	g kg^{-1} LW d^{-1}	g $kg^{-0.75}$ LW d^{-1}
Silage G	969	16.6	45.8
Silage H	1147	19.2	53.2
Dried grass	948	15.8	43.9
LSD	95.7	2.28	5.78
Significance	**	*	*

Table 7.8 Digestibility coefficients

	Dry matter	Gross energy	Organic matter	Total N
Silage G	0.727	0.727	0.746	0.764
Silage H	0.747	0.741	0.761	0.740
Dried grass	0.676	0.668	0.700	0.713
LSD	0.0199	0.0181	0.0178	0.0196
Significance	NS	NS	NS	*

Table 7.9 Concentrations of digestible and metabolisable nutrients

	DOMD ($g\ kg^{-1}$ DM)	DE ($MJ\ kg^{-1}$ DM)	ME ($MJ\ kg^{-1}$ DM)	DCP ($g\ kg^{-1}$ DM)
Silage G	677	14.0	11.4	124
Silage H	693	14.0	11.7	111
Dried grass	653	12.6	10.1	127
LSD	16.8	0.34	0.53	3.3
Significance	NS	NS	NS	***

Table 7.10 Retention of N and the excretion of N and energy in the urine

	N retention ($g\ d^{-1}$)	Urinary excretion (% of intake)	
		N	GE
Silage G	3.28	64.3	5.36
Silage H	5.23	55.1	4.15
Dried grass	4.68	54.0	5.47
LSD	3.28	11.90	1.755
Significance	NS	NS	NS

7.3.4 Rate of flow of digesta

Consumption of dry matter (Table 7.11) was slightly higher for Silage H than for Silage G, although the effect was not significant when expressed per unit body weight ($P > 0.05$). The reconstitution factor and the recovery of Ru and Cr administered in the marker solution, are presented in Table 7.12. There were no significant differences in the value of R or in the recovery of Cr in the faeces. Recovery of Ru was lower ($P < 0.05$) for Silage G than for Silage H or for dried grass. Rates of flow of components of digesta are expressed in absolute terms in Table 7.13, and related to intake of the components in Table 7.14. None of the differences between the silages attained statistical significance ($P > 0.05$). Two of the three terms indicating the extent to which nitrogenous components of the diet had been degraded in the rumen (Table 7.15) showed that the process was more extensive for Silage G than for Silage H or dried grass ($P < 0.05$).

The proportion of digestible organic matter which was apparently digested in the rumen is given in Table 7.16. The apparent efficiency of synthesis of microbial N expressed both per unit DOMR and per unit intake of ME are presented in the same table. Finally, in Table 7.17, three different terms describing the balance between nitrogenous components and organic matter in the diets, are given.

In Figure 7.5, the relationship between intake and abomasal flow of non-ammonia N is shown.

Table 7.11 Amount of dry matter consumed during measurement of digesta flow rate

	Dry matter intake		
	g d^{-1}	g kg^{-1} LW d^{-1}	g $kg^{-0.75}$ LW d^{-1}
Silage G	999	17.2	47.4
Silage H	1089	18.1	50.4
Dried grass	961	16.0	44.6
LSD	63.2	1.67	4.02
Significance	*	NS	NS

Table 7.12 Reconstitution factor 'R' and recovery of administered Ru and Cr

	Reconstitution factor	Apparent recovery (%)	
		Ru	Cr
Silage G	0.048	85.4	89.0
Silage H	0.021	97.4	89.8
Dried grass	-0.191	103.5	90.7
LSD	0.3592	12.54	10.89
Significance	NS	*	NS

Table 7.13 Flow of nutrients at the abomasum

	Nutrient flow ($g\ d^{-1}$)				
	Dry matter	Organic matter	Total N	Non-ammonia N	Microbial N
Silage G	656	475	25.0	23.1	11.1
Silage H	698	528	28.0	26.5	10.2
Dried grass	667	526	29.9	27.7	10.5
LSD	107.4	91.1	4.89	4.80	2.93
Significance	NS	NS	NS	NS	NS

Table 7.14 Flow of nutrients at the abomasum, related to nutrient intake

	Nutrient flow				
	Organic matter ($g\ kg^{-1}$ OM intake)	Total N ($g\ kg^{-1}$ TN intake)	Non-ammonia N ($g\ kg^{-1}$ TN intake)	($g\ kg^{-1}$ NAN intake)	Microbial N ($g\ kg^{-1}$ TN intake)
Silage G	524	967	895	964	418
Silage H	532	1074	1015	1060	391
Dried grass	591	1108	1029	1039	385
LSD	80.7	150.3	150.7	154.3	91.7
Significance	NS	NS	NS	NS	NS

Table 7.15 Microbial N content of abomasal digesta and apparent degradability of dietary N

	Microbial N content of abomasal digesta ($g\ kg^{-1}$ total N)	Uncorrected degradability of dietary N	Corrected degradability of dietary N
Silage G	431	0.452	0.510
Silage H	381	0.318	0.377
Dried grass	346	0.308	0.332
LSD	81.0	0.1042	0.1283
Significance	NS	*	*

Table 7.16 Dietary content of DOMR and the efficiency of synthesis of microbial N

	Proportion of DOM apparently digested in the rumen	Dietary content of DOMR ($g\ kg^{-1}$ DM)	Efficiency of synthesis of Microbial N	
			($g\ kg^{-1}$ DOMR)	($g\ MJ^{-1}$ ME intake)
Silage G	0.638	432	26.1	1.00
Silage H	0.616	426	22.2	0.77
Dried grass	0.583	382	29.6	1.09
LSD	0.1125	73.8	9.6	0.26
Significance	NS	NS	NS	NS

Table 7.17 Concentration of non-ammonia N in the DOM and DOMR, and of degradable N in the DOMR

	Non-ammonia N		Degradable N
	(g kg^{-1} DOM)	g kg^{-1} DOMR)	(g kg^{-1} DOMR)
Silage G	35.6	56.6	31.1
Silage H	33.2	54.4	21.3
Dried grass	43.2	75.4	23.9
LSD	1.05	11.09	9.07
Significance	***	NS	*

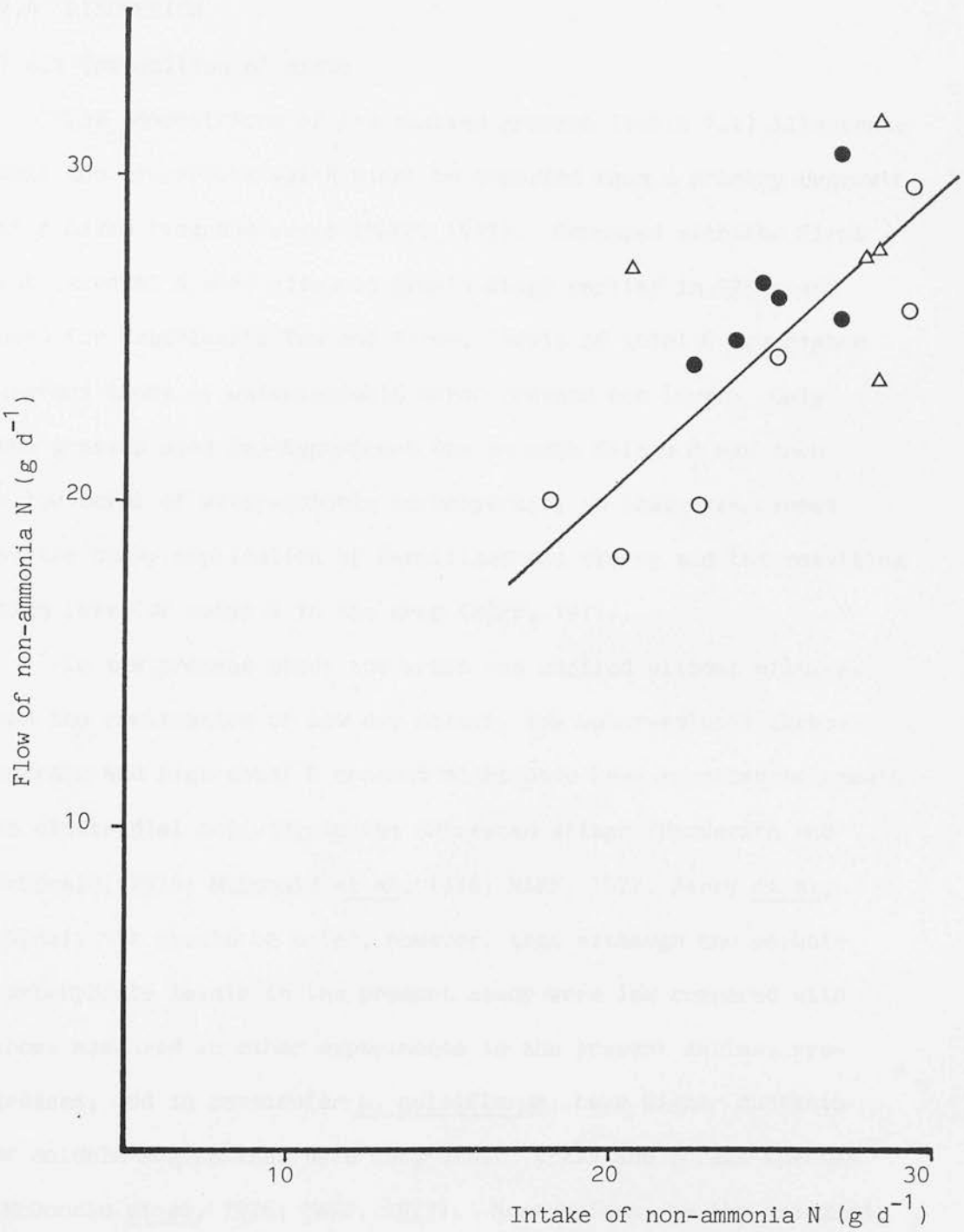

Figure 7.5 The relationship between the intake of non-ammonia N and the passage of that nutrient at the abomasum. Silage G (○), Silage H (●), dried grass (△)

line of best fit (silages only):

$y = 0.909x + 2.479$, $r = 0.8127$, $n = 12$

7.4 DISCUSSION

7.4.1 Composition of diets

The compositions of the ensiled grasses (Table 7.1) illustrate well the properties which might be expected from a primary regrowth of a mixed ryegrass sward (MAFF, 1977). Compared with the first cut taken at a more advanced growth stage earlier in 1981, and used for Experiments Two and Three, levels of total N are higher whereas those of water-soluble carbohydrate are lower. Only the grasses used in Experiment One to make Silage B had such a low level of water-soluble carbohydrate; in that case caused by the heavy application of fertiliser and slurry and the resulting high level of total N in the crop (MAFF, 1977).

In the present study the grass was ensiled without wilting, and the combination of low dry matter, low water-soluble carbohydrate and high total N content might have been expected to result in clostridial activity in the untreated silage (Henderson and McDonald, 1976; McDonald et al, 1976; MAFF, 1977; Barry et al, 1978a). It should be noted, however, that although the soluble carbohydrate levels in the present study were low compared with those measured in other experiments in the present series, ryegrasses, and in particular L. multiflorum, have higher contents of soluble sugars than have many other grass and forage species (McDonald et al, 1976; MAFF, 1977). Nonetheless, by the standards of its low pH, and low levels of butyrate and ammonia N, Silage G was well preserved (McDonald and Whittenbury, 1973; see Section 2.1.2.4 and compare Tables 4.2, 5.2 and 6.2). The inhibition of clostridia is to be expected in a silage where anaerobic conditions have been maintained, as was the case in the present

study, and where bacteria producing lactic acid have dominated the microflora. The high lactate, low acetate and low pH values confirm that this was the case in Silage G. The level of protein N in Silage G was higher than might be expected in an untreated grass silage (McDonald, 1982). The value of 452 g kg^{-1} total N was up to 47% higher than those observed for unwilted silages in Experiments One and Two. Whereas degradation of the soluble products of proteolysis is mainly attributable to clostridia, the proteolytic reactions themselves are considered to be catalysed by indigenous plant proteases (Kemble, 1956; Oshima and McDonald, 1978; McDonald, 1982). It has been demonstrated (McDonald, 1982) that these enzymes, although attenuated below pH 6.0, are still active at pH 4.0. Some proteolysis will therefore still occur after the 'instantaneous' fall in pH which follows the application of an acid additive to grass. The rate of fall of pH when grass is ensiled without treatment cannot be as high, and even when using an inoculum of lactic acid bacteria to assist the natural fermentation, Kemble (1956) found that pH 4.0 was not attained until three days after ensiling perennial ryegrass. Di Menna, Parle and Lancaster (1981) similarly found that a stable pH of 4.0 was not reached until about six days after ensiling untreated perennial ryegrass. Henderson and McDonald (1984) observed that untreated test-tube silage, maintained at 15-20^{o}C, had a pH of 5.20 48 h after ensiling. At this time the content of protein N was 580 g kg^{-1} total N. However similar silage which was kept at 30^{o}C for the same period had a pH of 4.35 and a protein N content of only 420 g kg^{-1} total N. Although the parameter was not determined, the value of 452 g protein N kg^{-1} total N for Silage G

was almost certainly much lower than that of the standing crop or the grass which was ensiled. Nevertheless it appears that several factors, including a vigorous natural fermentation and a rapid fall in pH, limited the activity of plant proteases.

Morgan et al (1980b), produced an unwilted ryegrass silage with similar concentrations of dry matter and total N to those of Silage G (169 g kg^{-1} and 24.8 g kg^{-1} DM) which had a much lower content of protein N (286 g kg^{-1} total N). Ammonia N and butyrate levels were higher than in Silage G however, and some of the proteolysis may have been due to clostridial activity. McDonald et al (1983) found that the level of protein N in ryegrass silage, made in test tubes with no pre-ensiling treatment, was only 260 g kg^{-1} total N. In this case, however, the fermentation had been dominated by heterofermentative lactic acid bacteria, acetate was the principal fermentation acid and the pH was relatively high (pH 4.9). Levels of butyrate and ammonia N (0.43 g kg^{-1} DM and 127 g kg^{-1} total N) were not indicative of proliferation of clostridia, although the ammonia N value prevented the silage from receiving a good rating for fermentation quality (McDonald and Whittenbury, 1973). The figures presented by Gill et al (1979) for silage made from perennial ryegrass and white clover are unusual. As in the present study, the protein N content was high (472 g kg^{-1} total N). However, with a pH of 4.5, and lactate and acetate concentrations of 36 and 31 g kg^{-1} DM respectively, it is clear that fermentation had not been as vigorous. The concentration of butyrate was not reported, but with an ammonia N content of 162 g kg^{-1} total N it is clear that clostridia were active.

Comparing the composition of Silage H with Silage G, it is clear that the formic acid and formalin had a slight restrictive effect on microbial fermentation. The concentration of acid products of fermentation (lactate + acetate + propionate + n-butyrate) was lower for Silage H (88.3 g kg^{-1} DM) than for Silage C (139.8 g kg^{-1} DM) although the difference in total acid content was smaller because of the high level of formic acid which remained in the treated silage. The large increase in ethanol content which was seen in Experiment Two, and was reported for many silages treated with formic acid in the Eurowilt studies (Zimmer and Wilkins, 1984), was not seen in the present experiment. It seems unlikely that the low level of formalin included in the additive could have had a pronounced effect on the activity of alcohol-producing yeasts. Large increases in ethanol content do not inevitably follow the use of formic acid, and McDonald et al (1983) found that the use of the additive at 4.9 l t^{-1} gave a silage with an ethanol content only 6.6 g kg^{-1} DM greater than that of an untreated control.

Because of its effect on plant proteases, formic acid alone would be expected to reduce proteolysis in the silo and give a silage with a higher protein content than that of an untreated control (Mcdonald, 1982). This was seen in Experiment Two, and in the present study the modest advantage of Silage H over Silage G (522 and 452 g kg^{-1} total N respectively) could have been accounted for entirely by the acid component of the additive.

The use, alone, of formalin at rates below about 4 l t^{-1} has frequently been associated with silages where proteolytic and saccharolytic clostridial activity has occurred (Wilkins et al,

1973; Castle et al, 1977; see Section 2.1.3.4.1). Castle et al (1977) used an additive containing formalin and concentrated sulphuric acid (66% and 15% respecively) at 2.0 and 4.4 l t^{-1}. At both rates there was evidence of clostridial fermentation which the mineral acid, presumably there for that purpose, did not arrest. In contrast, the mixture of formic acid with formalin (marketed as Silaform, BP Nutrition (UK) Ltd) allows the safe use of low rates of the latter because of the potent anti-clostridial effects of the former (Wilson and Wilkins, 1974; Zimmer and Wilkins, 1984). The levels of butyrate and ammonia N in Silage H indicate very low clostridial activity and confirm that any differential encouragement of clostridia by low levels of formalin can be completely controlled by the addition of formic acid. In contrast, Kaiser, Terry and Dhanoa (1981) found that clostridial activity was not controlled, when formic acid (as ADD-F, 2.0 l t^{-1}) and formalin were applied to ryegrass or red clover (Trifolium pratense) before ensiling, if the application rate of formaldehyde was less than 60 g kg^{-1} crude protein. The rate of application of formaldehyde in the present study was about 12 g kg^{-1} crude protein, but the rate of addition of formic acid was 80% higher than that used by Kaiser et al (1981).

7.4.2 Ruminal metabolism

All the foods were consumed satisfactorily in the periods when ruminal metabolism was being studied. With both silages, sufficient material was offered to allow a dry matter intake in excess of 1 kg d^{-1}. For the treated Silage H this was observed, although differences between sheep ensured that the 95% confidence interval of that silage's intake encompassed the target of 1 kg d^{-1}.

Because of the manner in which the food was offered, the observed intakes were not ad libitum and yet those of the silages are either very close to or greater than the appetite predicted by the Agricultural Research Council (ARC, 1980).

The concentrations of ammonia N in rumen liquor recorded for Silages G and H in the present study (Table 7.5, Fig 7.2) were the highest seen for silages in the whole series of experiments. This may reflect both the higher content of total N and the palatability of Silages G and H (see below). In absolute terms, however, the peak concentration which was attained when feeding dried grass was higher. When making comparisons between the diets, it is instructive to look not only at the absolute concentrations, which may be influenced by factors other than attributes of the foods, but also at the response of the ammonia concentration to ingestion of silage or dried grass. The first figure to be calculated (Table 7.18) is the maximum rise in concentration of ammonia N above the level recorded immediately before feeding, at 09.00 h. The high 'baseline' figures for dried grass ensures that this generated peak is higher for both silages than for the grass pellets. In Experiments Two and Three, this value was subsequently expressed per unit of nitrogen consumed in 24 h. Since the concentration of total N was high for Silages G and H, and close to that of the dried grass, this computation does not produce much change in the relative positions of the three diets in the present study. During the third period of this experiment the rate of consumption of each diet was measured by weighing the residue of food left by each sheep, at hourly intervals. In the two hours between offering the daily ration and attaining

Table 7.18 Ruminal concentration of ammonia N related to intake of nitrogen

	Silage G	Silage H	Dried Grass
1. Peak concentration of ammonia N ($mg\ l^{-1}$)	322	293	363
2. Concentration of ammonia N at 09.00 h ($mg\ l^{-1}$)	144	145	219
3. Generated ammonia N concentration (1.- 2.) ($mg\ l^{-1}$)	178	148	145
4. Intake of total N per day (g)	24.6	26.8	28.5
5. Generated ammonia N concentration (3. ÷ 4.) ($mg\ l^{-1}\ g^{-1}$ N)	7.2	5.5	5.1
6. Intake of total N between 09.00 and 11.00 h (g)	11.0	10.1	28.5
7. Generated ammonia N concentration (3.÷ 6.) ($mg\ l^{-1}\ g^{-1}$ N)	16.2	14.7	5.1

the maximum concentration of ammonia N in rumen liquor, the amounts of dry matter of each silage consumed did not differ significantly, and averaged 422g. In contrast, during the same period the whole daily allowance of dried grass was eaten. Thus the final figures presented in Table 7.18 indicate that the increase in ammonia N concentration, per unit N actually consumed up to the point when the maximum concentration was recorded, was much higher for the silages than for the dried grass. The value for Silage H was slightly lower than that recorded for the untreated Silage G

although the difference is small compared with that between the fermented and unfermented diets.

The final figures in Table 7.18 are persuasive evidence that the qualitative differences between the nitrogenous fractions of the silages and the dried grass greatly influence their susceptibility to microbial degradation in the rumen. The most obvious difference is their content of 'protein', which in the present study was considered to be nitrogenous material insoluble in hot water (Macpherson, 1952). This insoluble fraction consists mainly of intact plant proteins whereas the soluble compounds include peptides, amino acids, amines, nitrates and ammonia. Evidence from measurements of the disappearance of silage N from Dacron bags incubated in the rumen of steers (McDonald et al, 1983) indicates that much of the total N in an untreated silage is lost very rapidly. This initial loss can be reduced simply by increasing the concentration of protein N in the silage, although the effect is much greater if the protein is also protected by chemical binding with formaldehyde (McDonald et al, 1983).

Barry (1973a) found that formalin, applied at rates of 150 and 300 g HCHO kg^{-1} crude protein to field-dried hay, reduced levels of ammonia and volatile fatty acids in rumen liquor compared with those recorded when an untreated control was fed. Used at such high concentrations, it is likely that appreciable amounts of free formaldehyde persisted in the food (McDonald et al, 1983), and this may have acted directly to suppress the activity of the rumen microflora. Beever et al (1977) reported reduced concentration of ammonia N and more rapid attainment of the peak level, for ryegrass silage treated with formalin at 6.4 l t^{-1} than for an

untreated control. Again, these effects were obtained at an application rate of formaldehyde which was an order of magnitude higher than that used in the present study. Donaldson and Edwards (1979, 1980) made ryegrass silages treated with formaldehyde alone or with sulphuric or formic acid, at more modest levels (51 - 94 g HCHO kg^{-1} crude protein). When an untreated control silage was fed, the peak concentration of ammonia N in rumen liquor was about 470 mg l^{-1}. Formaldehyde alone and formaldehyde with formic acid produced silages with higher contents of protein N than that of the control (633, 722 and 420 g kg^{-1} total N respectively) whereas the effect of sulphuric acid with formaldehyde was less pronounced (580 g kg^{-1} total N). These differences in the composition of the nitrogenous fraction were broadly related to the ruminal ammonia concentrations observed when the silages were fed. Thus the sulphuric acid/formaldehyde treatment had the least effect, and the peak concentration of ammonia N was about 450 mg l^{-1}. The formaldehyde treated silage demonstrated a peak concentration of 350 mg ammonia N l^{-1}, and in common with the observations of Beever et al (1977) this peak was also attained earlier. The formic acid/formaldehyde treated silage, which had the lowest concentration of soluble 'non-protein' N induced the lowest concentration of ammonia N in rumen liquor (320 mg l^{-1}).

It thus appears that the concentration of ammonia N observed in rumen liquor depends both on the rate of supply, and on the solubility, of the nitrogenous fraction of the diet. In the present study (and in the others of the current series of experiments) the importance of the rate of supply of the nitrogen in the diet has been demonstrated by the high values recorded for the dried

grass, despite the insoluble nature of its nitrogenous components. The fact that the solubility of dietary nitrogen is also important is shown in all experiments by the much higher ammonia concentrations, per unit intake of N, from the silages. In the present study the small but significant differences between two silages with different contents of protein N concur with the proposed mechanism.

The diurnal patterns of rumen liquor pH (Table 7.6, Figure 7.3) were distinct for each diet. The pelleted, dried grass produced the expected pattern with a rapid drop in pH resulting from the fermentation of a large amount of water-soluble carbohydrate supplied within two hours after the food was offered. For much of the time when the pH was monitored, the value was lower when Silage H, rather than Silage G, was fed. The provision of more substrate for microbial fermentation, through more rapid consumption of dry matter, is unlikely to explain this difference. Intake over the 24 h period was only slightly higher for Silage H than for Silage G (see Table 7.4) and the measurements made during the third period (see above) suggested that the rate of consumption of dry matter was virtually identical for both silages. The amount of water-soluble carbohydrate supplied by the silages was quite different (0 and 64 g kg^{-1} DM for Silages G and H respectively), and this may, in part, be responsible for the observed difference in ruminal pH. Water-soluble carbohydrate will provide substrate for fermentation to volatile fatty acids immediately the diet is consumed, and it was not surprising, therefore, that the initial rate of fall of pH was greater for Silage G. However much of the volatile fatty acid production would be expected from the

actions of cellulolytic bacteria, for which substrate would be available in similar amount from each silage.

In the experiments described above, Donaldson and Edwards (1980) found that the pH was lower for all three treated silages than for the untreated control, 1 h after feeding. The silages treated with formaldehyde alone or formic acid and formaldehyde, maintained these more acid conditions throughout the steady-state 12 h period. After the initial drop however, the pH with the sulphuric acid/formaldehyde silage was not significantly different from that resulting from feeding the control silage. These differences seemed to be related to the water-soluble carbohydrate contents of the silages which were 151, 133, 64 and 41 g kg^{-1} DM for the formaldehyde, formic acid/formaldehyde, sulphuric acid/formaldehyde and untreated silages respectively.

The patterns of diurnal variation in the non-glucogenic ratio of the volatile fatty acids in strained rumen liquor are similar to those which were observed in previous experiments. In response to feeding, the NGR initially fell rapidly and then slowly regained its pre-feeding value during the rest of the day. The magnitude of the post-prandial fall was greater for Silages G and H than for those in Experiments Two and Three (compare Figures 5.4 and 6.4). In Experiment Two, the use of formic acid as a pre-ensiling additive dramatically increased the NGR whereas the use of the same additive with wilted grass had little effect in Experiment Three. In the present study, the use of formic acid with formalin appeared to increase slightly the ratio of non-glucogenic to glucogenic precursors in rumen liquor. This effect is similar to, although less pronounced than, that seen by Donaldson and

Edwards (1980). These authors found that the minimum NGR was about 0.6 units less when untreated, as opposed to formaldehyde or formic acid/formaldehyde-treated, silage was fed and concluded that this shift in NGR might reduce the efficiency with which dietary energy was used for growth (see Ørskov, 1975).

7.4.3 Dietary digestibility

During the periods of the trial in which dietary digestibility was being measured, the consumption of dry matter of Silage H was greater, both in absolute terms and per unit body weight, than that of the untreated control. The difference in mean intake was not much greater during this phase of each period than during the preceding one in which ruminal metabolism was measured (178 g d^{-1} and 172 g d^{-1} respectively) but the random variability associated with intake in the digestibility trial was smaller (cv% = 12.2 and 22.5 for intakes in Tables 7.7 and 7.4 respectively). Measurement of ad libitum intake was not one of the aims of the present study, and although many of the sheep were, in effect, being fed to appetite, some of the larger animals were not. Even with these constraints, the mean rate of intake of Silage H was, at 53.2 g DM $kg^{-0.75}$ LW d^{-1}, about 16% more than that considered by the Agricultural Research Council (ARC, 1980) to apply universally to silage eaten by sheep.

Variable effects of additives containing formalin, or formalin and acid, on the intake of the resulting silage, have been reported. Castle et al (1977) found that the ad libitum intake of silage by dairy cows was not different when formic acid or a mixture of formalin and sulphuric acid was added before ensiling. Crawshaw (1977) concluded that theuse of formalin alone as a pre-ensiling

additive would be likely to reduce the intake of silage. At low levels of formalin, a clostridial fermentation would be encouraged, and intake reduced by the high levels of butyrate, ammonia N and amines in the silage. Conversely with formalin applied at a sufficiently high rate to arrest fermentation, Crawshaw considered that reduced dietary digestibility might also reduce intake. Results reported by Barry et al (1978a) contradicted the conclusions of Crawshaw (1977), and demonstrated an increase in the voluntary intake of dry matter by sheep when lucerne silages were treated with formalin (8.0 l t^{-1}) before ensiling. These authors observed similar increases from the use of formic acid and were able to relate increased intake linearly to the reduction in intra-silo degradation of herbage protein. In the experiments described above (see Section 7.4.2), Donaldson and Edwards (1979, 1980) also found that formalin and acid/formalin additives produced silages which were more rapidly eaten by sheep than was an untreated control. In common with the findings of Barry et al (1978a), these workers showed that the effect on intake was related to the effect of the additive in reducing protein degradation in the silo. Thus formalin alone and formalin with formic acid, which were more efficient than formalin with sulphuric acid in reducing the degradation of herbage protein in the silo, increased dry matter intake by about 13% compared with 7.8% for the additive containing the mineral acid. In the present study, the intakes were higher in absolute terms, and the increase for the treated silage was also greater, being about 16% on a 'per unit metabolic liveweight' basis. However this important improvement was achieved with an appreciably smaller effect on the degradation of the herbage

protein. The protein N content of Silage H in the present study was 15% greater than that of Silage G whereas the formalin and formalin/formic acid silages of Donaldson and Edwards (1980) had protein N contents 72 and 58% higher respectively than that of the untreated control. In the present study, there were no consistent effects of the pre-ensiling additive used on the digestibility of dietary constitutents. There was a tendency for the dry matter, organic matter and gross energy of Silage H to be more digestible than the same components of Silage G, but the difference was never large enough to attain conventional statistical significance ($P > 0.05$). In contrast, the digestibility of total N was lower for Silage H than for Silage G ($P < 0.05$). The effects of formaldehyde in reducing the susceptibility of protein to degradation in the rumen, and often in lowering the apparent digestibility of the nitrogenous fraction over the whole gastro-intestinal tract, are well documented (Barry, 1973a; Barry, 1976; Beever et al, 1977; Barry, 1978a; Gill et al, 1979; Donaldson and Edwards, 1980). Were it not for the difference in total N content between Silages G and H, the use of formaldehyde with the latter, albeit at a comparatively low level, could have been proposed as the reason for the difference in the digestibility of their nitrogenous fractions. The influence of total N concentration on apparent digestibility has been discussed, and its effects observed, in the previous experiments of the present series (see Sections 4.4.2, 5.4.2 and 6.4.2). The difference in total N content was 57, 21 and 18% in Experiments One, Two and Three respectively. The corresponding reductions in digestibility for the silages with lower total N content were 9, 5 and 13%, indicating

that the effect on digestibility was not linearly related to the difference in nitrogen content. Silage G in the present study had a concentration of total N about 8% greater than that of Silage H, whereas the digestibility coefficient of the total N in the treated silage was 3% less than that of Silage G. It therefore seems likely that the formic acid/formalin additive, supplying 12 g HCHO kg^{-1} crude protein, had no detectable effect on apparent digestibility of silage N. In those experiments where digestion of the nitrogenous fraction has been reduced, the application rate of formaldehyde has been much higher, ranging from the modest rates of 51 to 94 g HCHO kg^{-1} crude protein used by Donaldson and Edwards (1979; 1980) to values of 150 and 300 g HCHO kg^{-1} crude protein tested by Barry (1973a).

There was a trend for urinary excretion of N and energy to be less for Silage H than for Silage G, but the differences were not significant ($P > 0.05$). Donaldson and Edwards (1980) in the experiments described above (see Section 7.4.2) found that the proportional loss of dietary energy in the urine was reduced when silage was treated with formic acid and formalin. Although more of the energy was lost in the faeces when this treated silage was fed in place of an untreated control, the greatly reduced loss of energy in the urine meant that the ME content of the treated silage was higher (12.29 vs 11.54 MJ ME kg^{-1} DM). This effect was not noted in the present study, although the loss of energy in the urine was much lower for Silage G (5.4% gross energy intake) than for the untreated silage fed by Donaldson and Edwards (8.9% gross energy intake) and therefore less amenable to manipulation.

The ME and DOMD contents of Silages G and H were high compared with the values for other silages in the present study, and in particular were higher than those for Silages C - F which were made from the first cut of the same pasture. These differences reflect the stage of growth of the crop when it was harvested, increasing maturity altering the ratio of fibre to more digestible components in the individual plants and hence reducing the digestibilities of dietary organic matter and energy (MAFF, 1977).

The higher digestibility of, and higher concentration of total N in, Silage G combined to make its DCP content significantly ($P < 0.001$) higher than that of Silage H. Thus any system using the concentration of digestible nitrogenous nutrients as the basis on which to decide the relative abilities of different diets to satisfy an animal's requirements for N, would consider Silage G to be superior to Silage H (Evans, 1960; Herland, 1982; Rohr, 1982).

7.4.4 Rate of flow of digesta

Intake of dry matter in the last week of each experimental period, when the rate of flow of digesta was being measured, remained high and close to the target of 1 kg dry matter d^{-1}. The mean intakes for Silage G and Silage H (47.4 and 50.4 g DM $kg^{-0.75}$ LW d^{-1} respectively) were higher than the Agricultural Research Council's suggested upper limit of 46 g DM $kg^{-0.75}$ LW d^{-1} (ARC, 1980) although in each case the 95% confidence interval encompassed the Council's value.

There was little evidence from the measured reconstitution factors (Table 7.12) to suggest that important sampling bias had occurred. However in this and the three previous experiments,

the coefficient of variation associated with the reconstitution factors was very high (CV% = 482), and the deviation of R from zero would not have been deemed statistically significant until after the point at which it had become biologically important. Recovery of Ru in the faeces was significantly lower for Silage G than for Silage H or the pelleted, dried grass ($P < 0.05$). There is no suggestion that differences in diet type directly influence loss of the marker from, or metabolism of the marker in, the gastro-intestinal tract. However differences in the amount of inorganic matter which is consumed, between diets or between animals, affects the ash content of the digesta which, in turn, alters the fluorescent response of the marker metal to irradiation with x-rays in the spectrophotometer (J MacKenzie, personal communication; see Section 8).

There were no differences between the silages in the rates of flow of the components of abomasal digesta, either in absolute terms (Table 7.13) or related to intake (Table 7.14). For total N, non-ammonia N and, to a lesser extent, organic matter, a trend of reduced apparent digestion of the component between the mouth and abomasum was noted for Silage H compared with Silage G. This shift in the site of digestion has been reported before as a consequence of the use of additives containing formaldehyde (Barry, 1973a; Beever et al, 1977; Gill et al, 1979). The relatively small effect noted in the present study reflects the low rate at which the formaldehyde was applied. The difference between the silages in flow of total N at the abomasum (per unit N intake), which may be interpreted as reduced ruminal digestion resulting from the use of the additive, is better explained as a consequence

of the difference between the silages in their content of total N. A reduction in flow of total N in response to increasing concentration of total N in the silage, has been noted before in the present study (see Sections 4.3.3 and 4.4.3) and, for different foods, by others (Thomas, 1982).

Because of its effect in reducing ruminal digestion, several workers have identified improved supply of nitrogenous nutrients to the small intestine as a benefit of the use of formaldehyde as an additive (Barry, 1976; Beever et al, 1977; Gill et al, 1979). Gill et al (1979) found that the flow of amino N, in g d^{-1} and g kg^{-1} total N intake, was much greater for silage treated with formaldehyde than for an untreated control. However the most striking effect of the additive on silage composition was a reduction in the degradation of herbage protein, and the intake of amino N was therefore higher when the treated silage was eaten. Flow of amino N at the duodenum was therefore 919 and 913 g kg^{-1} amino N intake for the untreated and treated silages respectively.

The concentration of microbial N in abomasal digesta was lower when Silage H, rather than Silage G, was eaten, although the difference was not statistically significant ($P > 0.05$, Table 7.15). However the ruminal degradation of nitrogenous components of the diet, whether or not corrected for the contribution of endogenous N to total N in the digesta, was less for Silage H than for Silage G ($P < 0.05$). Given the low application of formaldehyde, particularly in comparison with the rates used by other workers, this significant effect on the degradability of dietary N is perhaps surprising. However, Ohshima and Mcdonald (1978) suggested that a rate of 30 - 50 g HCHO kg^{-1} rumen degradable

protein was most likely to give improved intake and animal performance, avoiding the depressive effect of high rates of application on digestibility. Because of the comparatively low degradability of the total N in Silage G, the application rate of formaldehyde used in the present study was much higher per unit rumen degradable protein than per unit crude protein (30 and 12 $g\,kg^{-1}$ respectively), reaching the lower end of the desirable range identified by Ohshima and Mcdonald (1978).

The extent of the degradation of dietary N in the rumen and the net effect of the process on the supply of nitrogenous nutrients to the small intestine, forms an important part of several new systems for assessing the nutrient requirements of ruminant livestock. Such systems are under consideration in the United Kingdom (ARC, 1980; Alderman, 1982) and France (Journet and Verité, 1982). In other European countries in which DCP-based systems are still used attempts have been made to modify them to take account of recent findings regarding the effects of the balance between degradable and undegradable nitrogenous components of the diet on the supply of nutrients to the small intestine (Herland, 1982; Poppe and Gabel, 1982; Rohr, 1982).

The potential inadequacy of a system based on an assessment of the digestible nutrients in a food was clearly shown in the present study. The flow of non-ammonia N at the abomasum was the most reliable indicator of a nutrient supply measured (Oldham and Tamminga, 1980), and was slightly higher, both in absolute terms and per unit intake of non-ammonia N, for the treated Silage H. In contrast, Silage G was shown to have a slightly higher content of DCP ($P < 0.05$). Both interpretations of the relative worth of

the silages cannot be correct, and conclusive evidence would best be obtained by comparing the performance of productive animals against that predicted by the different rationing systems.

Before the publication of the Agricultural Research Council's new system (ARC, 1980), but after an interim report of the Council's Protein Group (Roy et al, 1977), Oldham, Broster, Napper and Smith (1979) measured the performance of 16 lactating Friesian cows offered four different rations which differed in their contents of rumen degradable and undegradable protein. All the diets contained 139 - 145 g crude protein kg^{-1} DM and the degradable: undegradable ratio was altered by changing the proportions of urea and fishmeal in the concentrate which was fed. Changing the nature of the dietary crude protein altered yields of milk fat and protein, liveweight gain and dry matter digestibility. Highest yields were combined with greatest liveweight gain when the ratio of degradable to undegradable protein was 2.5. For this ration, and the one in which the urea concentration was highest, measured output of protein above maintenance requirement was greater than that estimated factorially from the intake of digestible organic matter (Roy et al, 1977). In contrast, the two rations with lower degradable to undegradable protein ratios had measured outputs lower than those which were estimated.

Cooke (1985) described a series of field trials, studying the performance of dairy cows fed forage and one of two different compounded feeds. In each trial, the herd was divided into two groups balanced for lactation number, calving date and previous yield, with both groups having access to the same forage. One group received a compounded feed containing digestible undegradable

protein (DUPD) and rumen degradable protein (RDP) in the ratio 55:45, whereas the other was offered a ration of identical crude protein, energy, mineral, trace element and vitamin content, but with a DUDP:RDP ratio of 35:65. In three out of five trials, milk yield was higher in the group offered the less degradable compound. A potential link between response to undegradable protein and energy intake was seen in the two trials where no difference in milk yield was seen between the groups. In both cases the metabolisable energy content of the forage, fed ad libitum, was low (9.2 MJ ME kg^{-1} DM).

Tamminga (1982) concluded that, although the newer systems for evaluating the nutritional worth of dietary-protein were more accurate in concept and more precise in their discrimination between diets, they did not, when used with conventional ingredients, lead to any large improvement in the accuracy of predictions of animal performance. He was also conscious that even the newer evaluating systems paid little attention to the quality, or biological value of the nitrogenous nutrients supplied to the small intestine. Bergen, Purser and Cline (1967) demonstrated that there were important differences between strains of rumen bacteria in the digestibility and biological value of their amino acids. No information was available on variation in a mixed microbial population, but with variation between bacterial strains, the potential for differences in the quality of the microbial protein arriving at the ruminant small intestine exists. The quality of protozoal amino acids will also influence the biological value of the microbial fraction, and Harrison, Beever and Osbourne (1979) demonstrated that protozoal amino acids could contribute

up to 24% of total amino N flowing at the ovine duodenum.

In the most recent refinement of the protein evaluation system developed in the UK (ARC, 1984), it is concluded that total flow of amino acids at the duodenum will, in practice, be an accurate guide to the flow of essential amino acids. The Council are confident in this because of the rather constant composition of mixed microbial amino acids and the dominance, with normal rations, of this microbial fraction in duodenal digesta (ARC, 1980; 1984). The results of Thomas et al (1980) suggest that the second of these assumptions may not be correct. For four different silage diets, bacterial N made a relatively small contribution to total N at the duodenum. The amino acid profile of the duodenal digesta was therefore influenced directly by the profile in the diet.

There was no evidence from the results presented in Table 7.16 to suggest any change in the site of organic matter digestion due to the pre-ensiling additive. The figures for the proportion of DOM apparently digested in the rumen were within the ranges previously reported for forage diets (Sutton, 1971; ARC, 1980). They could not be distinguished statistically from the mean value, chosen by the Agricultural Research Council for inclusion in its factorial calculation of the nitrogen requirements of rumen microbes. The derived efficiencies of synthesis of microbial N showed no differences between diets ($P > 0.05$). The values for the silages, whether 'g N kg^{-1} DOMR' or 'g N MJ^{-1} ME^{-1}' were lower than the mean values for all diets suggested by the Agricultural Research Council but were in better agreement with the figures reported in ARC (1984) for grass silages (19.7 g N kg^{-1} DOMR and 0.71 g N MJ^{-1} ME intake). This highlights a problem of a factorial system of

dietary evaluation which attempts, for simplicity, to adopt mean values for its many factors. The approach is valid if the variation observed in the experimental results is not systematic and cannot, in part, be explained by classifying the results according to diet type (ARC, 1980). The data base used by the Council (ARC, 1984) does not contain results from different classes of diet in similar proportions and, in common with the experiments in the present study, the variability of the observations is high. Nevertheless, it seems inappropriate that grass silage (19.7 g microbial N kg^{-1} DOMR) and dried grass or legume forage (49.2 g microbial N kg^{-1} DOMR) should be considered to share a common value for efficiency of synthesis.

The small difference between the silages in their content of non-ammonia N (g kg^{-1} DOM, Table 7.17) was highly significant ($P < 0.001$). This reflects the derivation of the value, from the non-ammonia N content of silage dry matter, for which no variability term applied, and the DOMD which, in common with all the apparent digestibility measurements, was relatively precise. In contrast, there was no difference between Silage G and Silage H in their proportion of non-ammonia N to DOMR ($P > 0.05$).

In Figure 7.5, the simple relationship between intake and abomasal flow of non-ammonia N is shown. Despite the fact that there were slight differences between the silages in terms of non-ammonia N flow per unit intake, a single straight line equation can satisfactorily describe the data set for the silages. This agrees with the findings of the Agricultural Research Council (ARC, 1984), that intake of nitrogen alone was likely to be the best indicator of the flow of non-ammonia N to the small intestine, for silages.

7.5 CONCLUSIONS

Experiment Four confirmed the conventional characteristics of a primary regrowth of a ryegrass sward, with higher total N and lower water-soluble carbohydrate contents in the ensiled grass than in the first cut used in earlier experiments. The silages produced from this material, in turn, had higher contents of digestible and metabolisable nutrients.

It was apparent that at the levels of total N and water-soluble carbohydrate tested, stable unwilted silage could be made without the use of a pre-ensiling additive. The low pH and relatively high content of protein N in the untreated silage indicated that with ryegrass of this type buffering capacity was no impediment to vigorous fermentation, a rapid fall in pH and consequent restriction of proteolysis (Henderson and McDonald, 1984).

The use of formic acid with formalin, in the ratio 4:1, allowed the application rate of the aldehyde to be much lower than would be required to suppress clostridial activity if formalin was the sole constituent of the additive. When such a mixture was used at the rate of 4.5 l t^{-1}, fermentation and proteolysis were reduced but not stopped completely.

The trend shown in Experiments Two and Three, indicating greater production of ammonia N in the rumen for the silages than for the pelleted dried grass, were confirmed in the present study. The contrast was not apparent when the concentrations of ammonia N in absolute terms were compared, but rather was manifest as far higher production per unit intake of dietary N.

The comparatively low level of formaldehyde tested had only slight effects on the concentration of ruminal ammonia N, but

did significantly reduce the degradation of nitrogenous components of the diet between the mouth and abomasum. The fact that a low level of formaldehyde could achieve this highlights the potential for 'overprotection' which exists when formalin is used alone, of necessity at much higher rates.

Once more the conclusions of the Agricultural Research Council (ARC, 1984) were confirmed, and for the silages alone, intake of non-ammonia N proved to be an adequate estimator of the flow of that fraction at the abomasum.

8. CONCLUDING DISCUSSION

8.1 REPEATABILITY OF MEASUREMENTS

In the discussion sections of Experiments One to Four, frequent comparisons were made between the values of various parameters recorded for the silages in different experiments. These comparisons were encouraged by the relative constancy, irrespective of the experiment, of the values determined for any given parameter when dried grass was the diet. This repeatability may be gauged for any measurement by comparing the appropriate tables in Sections 4.3, 5.3, 6.3 and 7.3. It is illustrated for a range of measurements in Table 8.1 and Figure 8.1.

The coefficients of variation shown in Table 8.1 demonstrate that the importance of the variability between experiments was generally greater for those measurements for which levels of random variability within experiments was high.

The diurnal variation in ruminal ammonia N content (Figure 8.1) remained remarkably constant from experiment to experiment. The pattern of rumen liquor pH was also very predictable, but the variation between experiments in the diurnal changes in the NGR, was more marked (compare Figures 5.4, 6.4 and 7.4).

If there had been no variability within experiments for values determined when dried grass was the diet, and the present level of variation between experiments had occurred, it would have been sensible to attempt to correct all observations for the systematic effects of experiment number. However, in view of the normal levels of variation within each experiment, this approach would be inappropriate. A subjective assessment of figures such as those in Table 8.1 and Figure 8.1 does, however, suggest that the results from different experiments are sufficiently alike to allow uncorrected comparisons between them.

Table 8.1 Variability, between experiments, of selected measurements taken from sheep eating pelleted, dried grass

	Digestibility of total N	ME (MJ kg^{-1} DM)	Flow of total N (g d^{-1})	Reconstitution factor	Corrected degradability of dietary N	Efficiency of synthesis of microbial N (g kg^{-1} DOMR)
Mean	0.711	10.0	30.2	- 0.189	0.339	28.9
Range	0.693 to 0.735	9.7 to 10.4	27.3 to 33.0	- 0.233 to - 0.165	0.262 to 0.452	26.3 to 34.7
SD	0.0178	0.3162	2.351	0.0134	0.0799	4.385
CV%	2.51	3.16	7.78	16.59	23.60	15.20

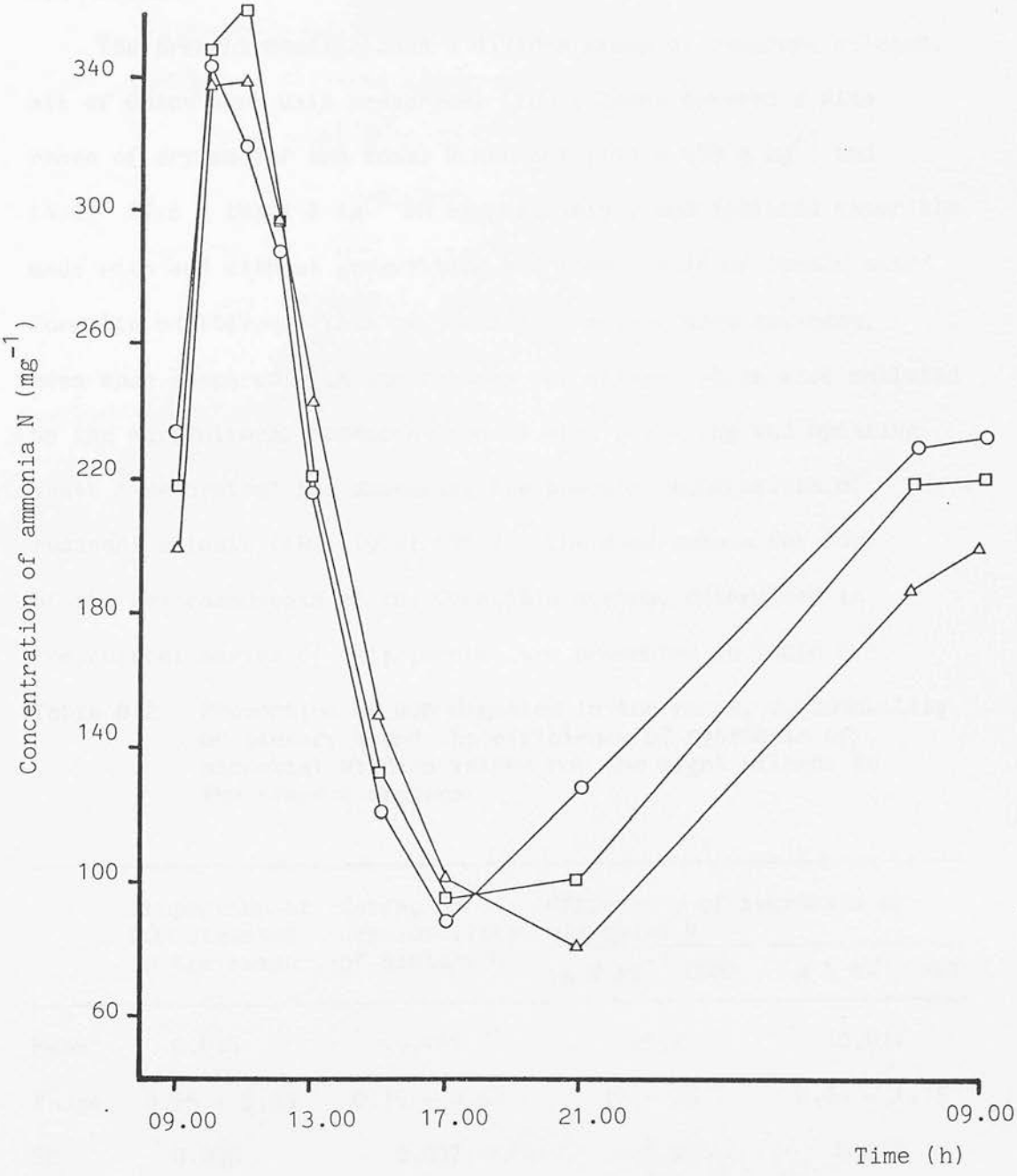

Figure 8.1 Diurnal variation in the concentration of ammonia N in strained rumen liquor from sheed fed dried grass once daily at 09.00 h. Experiment Two (○), Experiment Three (△), Experiment Four (□)

8.2 SILAGES

The present studies used a diverse group of ryegrass silages, all of which were well preserved. The silages covered a wide range of dry matter and total N content (190 - 438 g kg^{-1} and 14.0 - 29.6 g total N kg^{-1} DM respectively), and included materials made with and without pre-wilting and formic acid or formic acid/formalin additives. This represents an appreciable database, even when compared with the figures for silages which were collated by the Agricultural Research Council when preparing and updating their 'New System' for assessing the protein requirements of ruminant animals (ARC, 1980; 1984). The mean values for four of the key components of the Council's system, determined in the current series of experiments, are presented in Table 8.2.

Table 8.2 Proportion of DOM digested in the rumen, degradability of dietary N and the efficiency of synthesis of microbial N: Mean values for the eight silages in the present studies

	Proportion of DOM digested in the rumen	Corrected degradability of dietary N	Efficiency of synthesis of microbial N	
			(g N kg^{-1} DOMR)	(g N MJ^{-1} ME)
Mean	0.614	0.467	26.2	0.917
Range	0.55 - 0.69	0.35 - 0.63	17 - 35	0.64 - 1.15
SD	0.038	0.097	6.61	0.210
CV%	6.2	20.8	25.2	22.9

8.2.1 The proportion of DOM digested in the rumen

In 1980, the Agricultural Research Council (ARC, 1980) decided that a single value of 0.65 would be an adequate approximation, over all diet types eaten by cattle and sheep,

for the proportion of digestible organic matter which was apparently digested in the rumen. Only two measurements of the parameter for silage eaten by sheep were included in the Council's database. The average of these was 0.62. In a subsequent revision (ARC, 1984), the overall mean value from all the studies collated by the Council was again 0.65. On this occasion, five values of the parameter determined for silage in sheep were included, the mean and estimated standard deviation being 0.65 and 0.048 respectively. The mean value from the present studies (0.614, SD = 0.038) agrees reasonably with these previously published estimates. However the Agricultural Research Council's most recent conclusions (ARC, 1984) recommended that a value of 0.68 be adopted when silage was fed as the sole component of a ruminant diet, and Thomas (1982) concluded that the value was almost constant at 0.70 ± 0.01, $n = 17$. The discrepancy is best explained by inter-species differences, the value being appreciably higher (0.73) when silages are fed to cattle (ARC, 1984). The importance of these differences must, however, be kept in perspective, and an increase of 0.1 in the proportion of DOM apparently digested in the rumen would only increase the supply of microbial N to the duodenum by about 1 g d^{-1} for the sheep used in the present studies.

The fact that the proportion of DOM digested in the rumen could be determined quite precisely, and that the mean value in the present studies agreed with previous observations, provides circumstantial evidence that the technique used to measure the rate of flow of digesta was successful. Although conventional statistical significance could not always be ascribed to deviations

of the reconstitution factor from zero, the tendency for the value to be negative throughout the series of experiments suggested that systematic sampling bias did occur and that the dual-phase marker technique was required.

The use of Ru-P and Cr-EDTA has disadvantages which are mainly associated with the equipment required to measure non-radioactive Ru. Only two x-ray fluorescence spectrophotometers were available at agricultural research facilities in the United Kingdom in 1981. The fluorescent response of Ru or Cr in biological samples is affected by the mineral (ash) content of the sample. Increasing the ash content attenuates the response and, if not corrected for, leads to underestimation of the concentration of the marker metal in the sample. The problem may be avoided by ensuring that the marker-free blank has the same ash content as the marked sample or by the inclusion of an internal standard in the material immediately before analysis (J Mackenzie, personal communication). The first solution was adopted in the present studies, and is appropriate for animals kept in the controlled environment of a metabolism cage. For grazing animals, the possibility that individuals will differ in their intake of soil means that the second option is favoured.

The use of two markers, taking account of the solid and liquid phases of digesta, has now become well established in nutritional research. The technique has spread beyond conventional work with domesticated sheep and cattle, and Dierenfeld, Hintz, Robertson, Van Soest and Oftedal (1982) used unbroken wheat grains and polyethylene glycol to mark, respectively, the solid and liquid phases of the digesta of two Giant Pandas. Alternatives

to the combination of Ru-P and Cr-EDTA, such as rare-earth metals (Crooker, Clark and Shanks, 1982) and Cr_2O_3 with Cr-EDTA (Harrison, Laby and Mangan, 1982) continue to be studied.

With present technology, the rate of flow of digesta to the small intestine can only be measured in fistulated animals. The problems presented by the use of such surgery are both practical and conceptual. In the present studies, little ill health or apparent discomfort was inflicted on the sheep as a result of their rumen cannulas. Cannulation at this site is generally considered to be reliable and trouble free (Balch and Cowie, 1962; macrae and Wilson, 1977). In contrast, the abomasal cannulas gave cause for concern on numerous occasions and necessitated the maintenance of a group of animals twice the size of that needed for experimentation at any one time. Catheterisation of the abomasum has been shown to cause no disruption to the activity of the gastrointestinal tract (Wenham and Wyburn, 1980) but the problems of maintaining sheep in a healthy state once the gastrointestinal tract posterior to the reticulo-rumen has been fistulated, should not be underestimated (Macrae, Reid, Dellow and Wyburn, 1973; Smith and White, 1982).

The applicability to the whole ruminant population, of the values obtained using surgically modified sheep, is difficult to assess. The normal, productive, functioning of cannulated animals is encouraging (Oldham, Bruckental and Nissenbaum, 1980; Cooke, 1985). However, observation of altered energy metabolism in response to fistulation surgery (Macrae et al, 1982) indicates that extrapolation from cannulated to intact animals may, in some cases, be unwise.

8.2.2 Degradability of dietary nitrogen

The mean value determined in the present studies for the degradation of the nitrogenous components of silage between the mouth and the abomasum (0.467) was lower than most previously published estimates (ARC, 1980; Thomas, 1982). However, Thomas et al (1980) observed that bacterial N made a relatively small contribution to duodenal crude protein when silages were fed to sheep, and therefore concluded that the extent of degradation of silage N was low.

Underestimation of the extent of the degradation of dietary N could arise if the technique used did not measure the nucleic acid in digesta quantitatively. However the modified method of Guinn (1966) appeared, after considerable initial difficulty, to work satisfactorily. The use of freeze-drying would be expected to avoid the variable losses of nucleic acid which can occur after repeated freezing and thawing of wet samples (Guinn, 1966; McAllan and Smith, 1969; Brennan, Butters, Cowell and Lilly, 1979). The fact that the proportion of DOM apparently digested in the rumen, and the efficiency of synthesis of microbial N (g kg^{-1} DOMR) determined for silages in the present studies, agree well with previous estimates, also indicates that the assessment of microbial N, and hence the degradability of dietary N, was accurate.

The most recent revision of the Agricultural Research Council's system for protein evaluation (ARC, 1984) moves away from the assessment of true degradability (the survival of intact dietary protein at the duodenum) and instead reviews values for the degradation of the nitrogenous components of foodstuffs

incubated in synthetic fibre bags in the rumen. The move reflects the difficulties of making rapid, accurate measurements *in vivo*, and also the recognition that the values determined *in vivo* pertain only to the specific conditions under which they are obtained (Siddons and Paradine, 1981; ARC, 1984). Nevertheless, it is still important that the relationship between *in sacco* or *in vitro* measurements, and those determined *in vivo*, is known (Broderick, 1982; ARC, 1984). For this, accurate *in vivo* techniques are required. Improvements continue to be made in methods for detecting specific microbial amino acids in intestinal digesta, and DAPA, lysine and leucine have all been recently suggested as suitable endogenous markers (Theurer, 1982). More rapid extraction and precipitation techniques have been suggested for determining the nucleic acid content of digesta, (Ben-Ghedalia, 1981) but the development of a method for measuring purine and pyrimidine bases, using high performance liquid chromatography, appears to offer the biggest improvement in the use of microbial nucleic acid as an endogenous marker (Schelling, 1982).

8.2.3 Efficiency of synthesis of microbial nitrogen

The Agricultural Research Council's original proposal suggested that a mean value of 30 g N kg^{-1} DOMR be adopted for the efficiency of synthesis of microbial N. Only two values determined with silages were included, and these spanned a wide range (15 - 45 g N kg^{-1} DOMR). Subsequently (ARC, 1984) the overall mean was amended to 32 g N kg^{-1} DOMR, using a database which included nine measurements of silages eaten by sheep (mean = 19.7, SD = 6.80). However the Council concluded that a standardised value of 23 g N kg^{-1} DOMR be adopted when silage was the sole

constituent of ruminant diets since the mean value determined for cattle was 26.8 g N kg^{-1} DOMR. Thomas (1982) collated results from 12 experiments in which silages were fed to sheep and found that the mean efficiency of synthesis of microbial N was 21.1 g N kg^{-1} DOMR (SD = 6.9).

The mean value from the present study (26.2 g N kg^{-1} DOMR) is in reasonable agreement with those previously observed, although it indicates a slightly higher efficiency. This reinforces the opinion that the lower apparent degradability of dietary N was not the result of incomplete measurement of the microbial fraction.

The preferred term for the efficiency of synthesis of microbial N is now g N MJ^{-1} ME intake (ARC, 1984). The Council's suggested averages for efficiency of synthesis (23 g N kg^{-1} DOMR) and proportion of DOM apparently digested in the rumen (0.68) are slightly different from those determined in the present studies and combine to imply a value of about 1.0 g N MJ^{-1} ME intake (ARC, 1984). The Council also cite the results of five experiments in which the efficiency of microbial synthesis per unit intake of ME was measured directly, the average being 0.71 g N MJ^{-1} ME. The mean value from the present study was 0.92 (SD = 0.21), agreeing well with the derived, and moderately well with the measured, values.

8.2.4 Relationships between intake and nutrient supply

Thomas (1982) demonstrated a highly significant negative relationship between the concentration of total N in the DOM and the apparent gain or loss of nitrogen between the mouth and duodenum of sheep eating dried forages. At low concentrations of total N there was an apparent gain of nitrogen between the

mouth and duodenum, whereas at high concentrations flow of nitrogen was less than that consumed. For hays, nitrogen passage at the duodenum equalled nitrogen intake when the dietary concentration was about 41 g total N kg^{-1} DOM. Thomas (1982) could not fit a similar relationship to his collated data on silages, and instead found a simple positive linear relationship between intake and duodenal flow of total N. This relationship was most satisfactory for additive-treated silages, and the fit was much poorer when the results from untreated silages were included. In the present series of experiments, the relationship between intake (X) and abomasal flow (Y) of total N was poorer than that identified by Thomas (1982):

$$Y = 0.408X + 13.753 \; ; \; r = 0.675, \; n = 8$$

This confirms that in silages, intake of nitrogen is a poor indicator of passage of the component to the small intestine. In Figure 8.2, the results from Silages A - H are presented in the same manner as that used by Thomas (1982) for dried forages. A strong negative relationship exists between the concentration of total N in dietary DOM and the apparent efficiency with which dietary N passes to the abomasum. Interestingly, in view of the reputation for the nitrogen in silage to be poorly utilised, the concentration of total N above which there is a net loss of nitrogen between the mouth and abomasum, is lower for the silages in Figure 8.2 (35 g kg^{-1} DOM) than for the dried forages described by Thomas (1982).

Abomasal or duodenal flow of non-ammonia N is a better empirical estimator of the supply of amino N to the small intestine than is flow of total N (Oldham and Tamminga, 1980). In Figure 8.3,

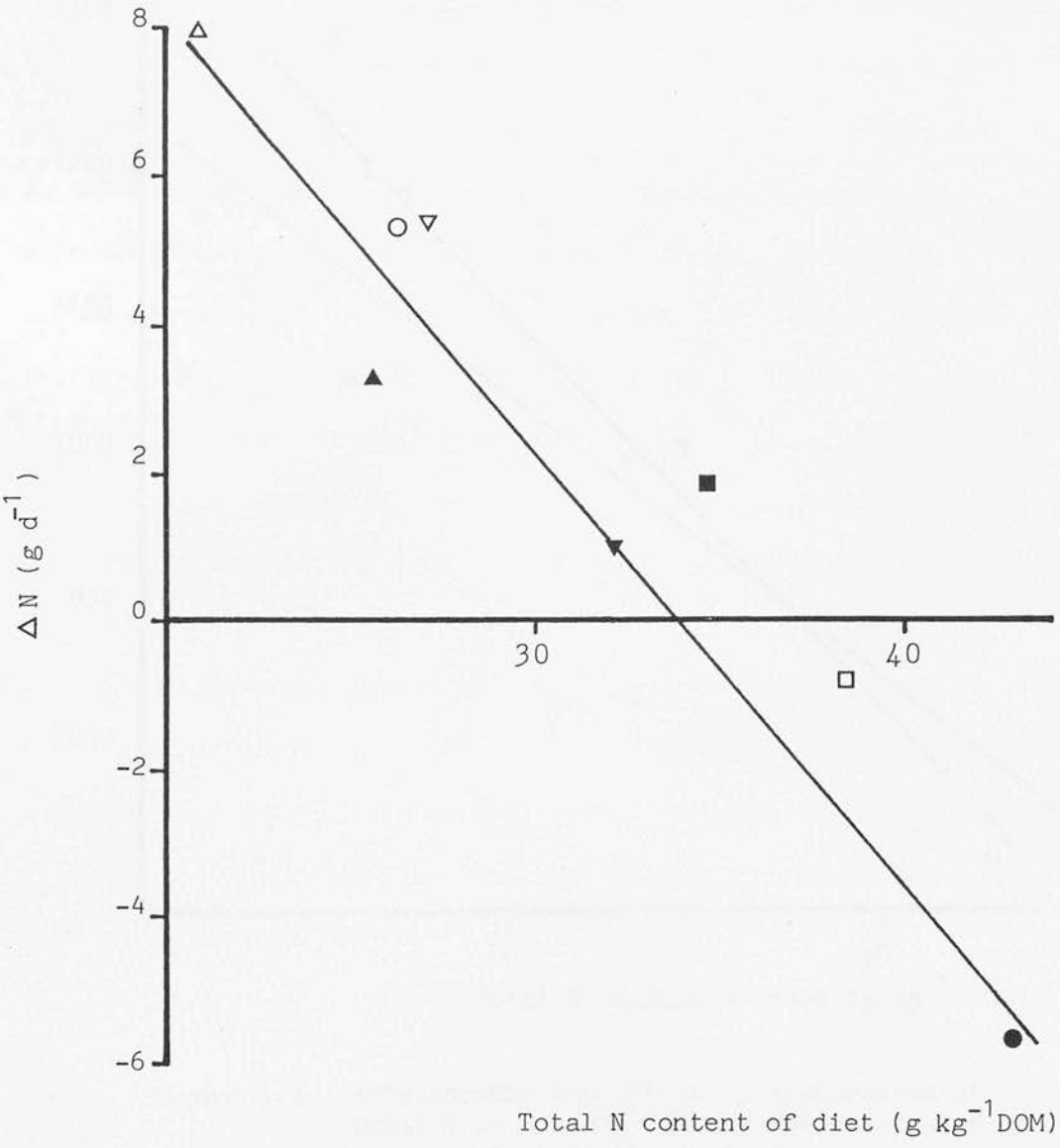

Figure 8.2 The relationship between the concentration of total N in dietary DOM and the difference (Δ N) between daily passage of total N at the abomasum and N intake. Silage A (○), Silage B (●), Silage C (△), Silage D (▲), Silage E (▽), Silage F (▼), Silage G (□), Silage H (■)

$y = 19.545 - 0.577x$; $r = -0.9587$, $n = 8$

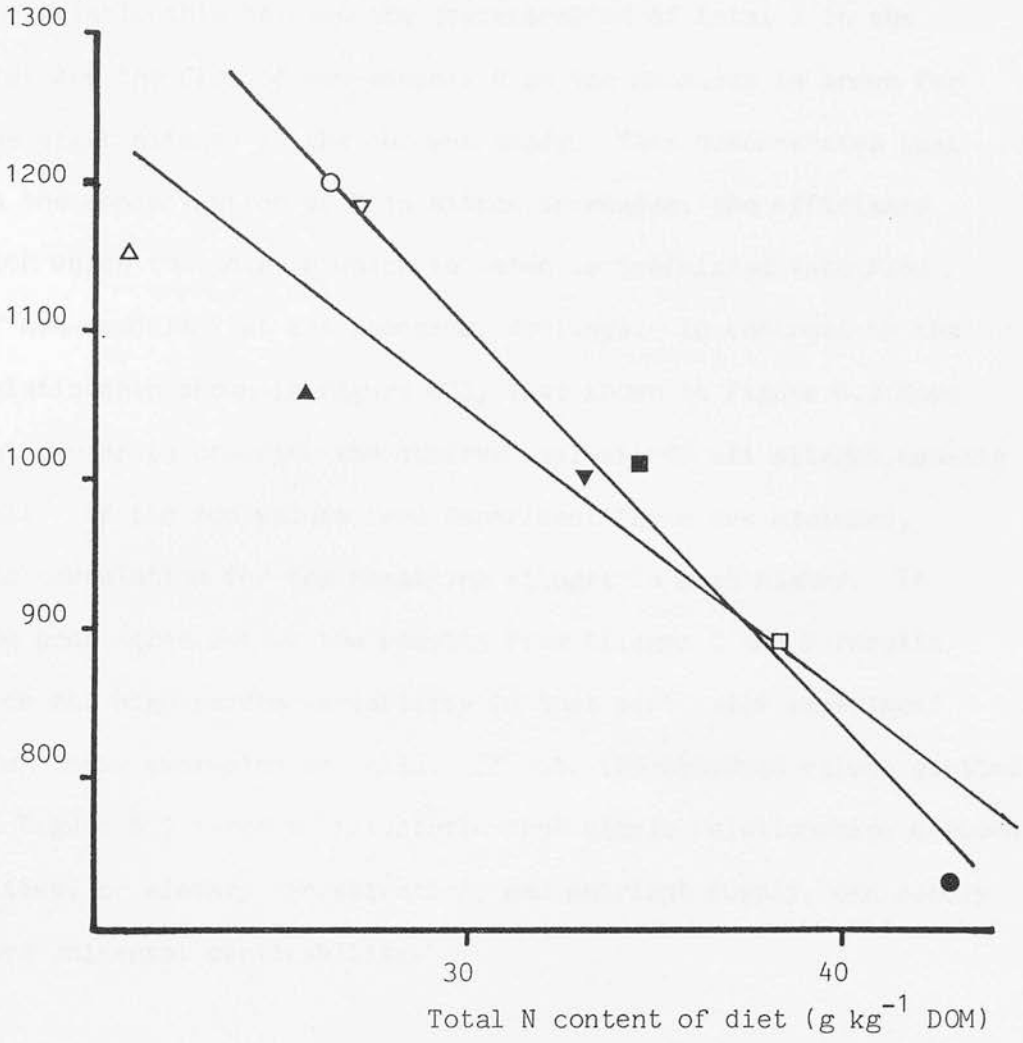

Figure 8.3 Relationship between the concentration of total N in dietary DOM and the passage of non-ammonia N at the abomasum.
Silage A (○), Silage B (●), Silage C (△), Silage D (▲), Silage E (▽), Silage F (▼), Silage G (□), Silage H (■)

$y = 1639.72 - 19.633x$, $r = -0.9102$, $n = 8$

($y = 1912.86 - 27.021x$, $r = -0.9878$, $n = 6$ when Silages C and D are excluded)

the relationship between the concentration of total N in the diet and the flow of non-ammonia N at the abomasum is shown for the eight silages in the current study. This demonstrates that as the concentration of N in silage increases, the efficiency with which the total N which is eaten is translated into flow of non-ammonia N at the abomasum, declines. In contrast to the relationship shown in Figure 8.2, that shown in Figure 8.3 does not appear to describe the observed values for all silages equally well. If the two values from Experiment Three are excluded, the correlation for the remaining silages is much higher. If the poor agreement of the results from Silages C and D results from the high random variability in that particular experiment, then their exclusion is valid. If not, the observed values plotted in Figure 8.3 serve to illustrate that simple relationships between intake, or dietary concentration, and nutrient supply, can rarely have universal applicability.

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APPENDIX A

ESTIMATION OF DIGESTA FLOW RATE

The dual phase marker system, used in the present studies to estimate the rate of flow of digesta at a specific point in the animal's gastrointestinal tract, is an indirect method of assessment. All such indirect methods rely on the principle of estimation of the flow rate of digesta by reference to the dilution of known amount of an indigestible marker substance in true digesta. True digesta (TD) may be thought of as the material contained within the lumen of the digestive tract. When an exogenous marker is administered at a measured rate and, once equilibrium has been established, is observed to have a certain concentration in the true digesta flowing through the gastrointestinal tract, the rate of flow of digesta may be readily calculated (equation 1).

Marker infusate $x\ g\,d^{-1}$

Digesta marker concentration $y\ g\ kg^{-1}$

Digesta flow rate $= \frac{x}{y}\ kg\ d^{-1}$ (equation 1)

The major disadvantage of the simple 't-piece' cannula is its inability to allow the reliable acquisition of a digesta sample containing solids and liquids in the same proportions as those of true digesta within the tract. Thus, a representative sample of true digesta cannot be obtained. This problem may be elegantly resolved using a dual-phase marker system (Faichney, 1975b), itself a modification of the double marker technique proposed by Hogan and Weston (1976), which employs two exogenous markers whose distributions between the solid and liquid phases of digesta differ at equilibrium. Explanation of the mathematical manipulations performed to express

the flow rate of digesta given the initial SD, CD and marker infusate figures, will be facilitated using the following nomenclature:

Ru I	=	ruthenium infusate (gd^{-1})
Cr I	=	chromium infusate (gd^{-1})
$[Ru]_{SD,CD,TD}$	=	ruthenium concentrations in sampled digesta, centrifuged digesta and true digesta ($g\ kg^{-1}$)
$[Cr]_{SD,CD,TD}$	=	chromium concentrations in sampled digesta, centrifuged digesta and true digesta ($g\ kg^{-1}$)
$Ru_{SD,CD,TD}$	=	ruthenium concentrations in sampled digesta, centrifuged digesta and true digesta expressed as a percentage of the daily ruthenium infusate
$Cr_{SD,CD,TD}$	=	chromium concentrations in sampled digesta, centrifuged digesta and true digesta expressed as a percentage of the daily chromium infusate

As has been illustrated in equation (1) the TD flow may be expressed as follows:

$$\text{TD flow} = \frac{\text{Cr I}}{[\text{Cr}]\ \text{TD}}$$

or

$$\text{TD flow} = \frac{\text{Ru I}}{[\text{Ru}]\ \text{TD}}$$

By inspection, it can be seen that the ratios Cr I:Cr_{TD} and Ru I:Ru_{TD} must be the same. Alternatively, this can be viewed as the concentrations of ruthenium and chromium in TD being the same when each is expressed as a proportion of its own daily infusate. Thus Cr_{TD} must equal Ru_{TD}. It is observed, however, that the same condition does not apply to the corresponding concentrations in SD, and Cr_{SD} does not (usually) equal Ru_{SD}. It can therefore be seen that the simple cannula is indeed functioning imperfectly and that the material which issues from it is not a representative

sample of digesta within the lumen of the tract.

If a portion of the non-representative SD is centrifuged, two fractions, a supernatant and a pellet of CD, will be produced. Both these fractions will contain ruthenium and chromium in concentrations and proportions different to those observed in the SD. One can now envisage that a given amount (y) of this centrifuged digesta (or the supernatant) could be added to, or removed from, a given amount (x) of the sampled digesta to produce a composite containing the ruthenium and chromium in the correct, true digesta, proportions (equation 3).

$$x\ Cr_{SD} + y\ Cr_{CD} = Cr_{TD} = Ru_{TD} = x\ Ru_{SD} + y\ Ru_{CD} \qquad (3)$$

The ratio $\frac{y}{x}$ may now be evaluated (equation 4)

$$\frac{y}{x} = \frac{Ru_{SD} - Cr_{SD}}{Cr_{CD} - Ru_{CD}} = R \qquad (4)$$

Where 'R' is the reconstitution factor, the amount of centrifuged digesta which must be added to, or removed from, one unit of sampled digesta to reconstitute true digesta. This reconstitution could, in theory, be performed physically but problems would obviously arise with a negative 'R' value and the concomitant problem of removal of centrifuged digesta from sampled material. The task is therefore undertaken mathematically (equation 5).

$$\frac{[Cr]_{SD} + R\ Cr_{CD}}{1 + R} = [Cr]_{TD} \qquad (5)$$

$$\frac{[Ru]_{SD} + R\ Ru_{CD}}{1 + R} = [Ru]_{TD}$$

The rate of flow of true digesta may now be evaluated using either of the calculated true digesta marker concentrations (equation 2). It can be seen that while both markers are important in ascertaining the degree of correction which must be applied

to the sampled digesta, one or other becomes redundant once the reconstitution factor has been determined.

Once the rate of flow of TD has been established, the rate of flow of any component of that digesta may be calculated from the known concentrations of the component in the SD and CD fractions. These concentrations are substituted for those of the flow rate marker in equation 5 and the resultant TD concentrations are multiplied by the previously evaluated TD flow.

These principles are illustrated in the following worked example. The figures apply to sheep 487 consuming the dried, pelleted, grass diet in period two of Experiment Four.

$Ru\ I = 0.0182\ gd^{-1}$

$Cr\ I = 0.2528\ gd^{-1}$

$[Ru]_{SD} = 0.023\ g\ kg^{-1}DM;\ Ru_{SD} = 126.247$

$[Ru]_{CD} = 0.033\ g\ kg^{-1}DM;\ Ru_{CD} = 181.138$

$[Cr]_{SD} = 0.340\ g\ kg^{-1}DM;\ Cr_{SD} = 134.481$

$[Cr]_{CD} = 0.175\ g\ kg^{-1}DM;\ Cr_{CD} = 69.218$

Thus:

$$R = \frac{(126.247 - 134.481)}{(69.218 - 181.138)} = 0.07357$$

$$[Cr]_{TD} = \frac{0.340 + (0.07357 \times 0.175)}{(1 + 0.07357)} = 0.3287\ g\ kg^{-1}DM$$

$$[Ru]_{TD} = \frac{0.023 + (0.07357 \times 0.033)}{(1 + 0.07357)} = 0.0237\ g\ kg^{-1}DM$$

$$\frac{CrI}{[Cr]_{TD}} = \frac{0.2528}{0.3287} = \frac{RuI}{[Ru]_{TD}} = \frac{0.0182}{0.0237} = 0.769\ kg\ DM\ d^{-1}$$